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Reduction of Toxicity to Aquatic Organisms by Industrial Wastewater Treatment

(U.S.) EG and G, Bionomics
Wareham, MA

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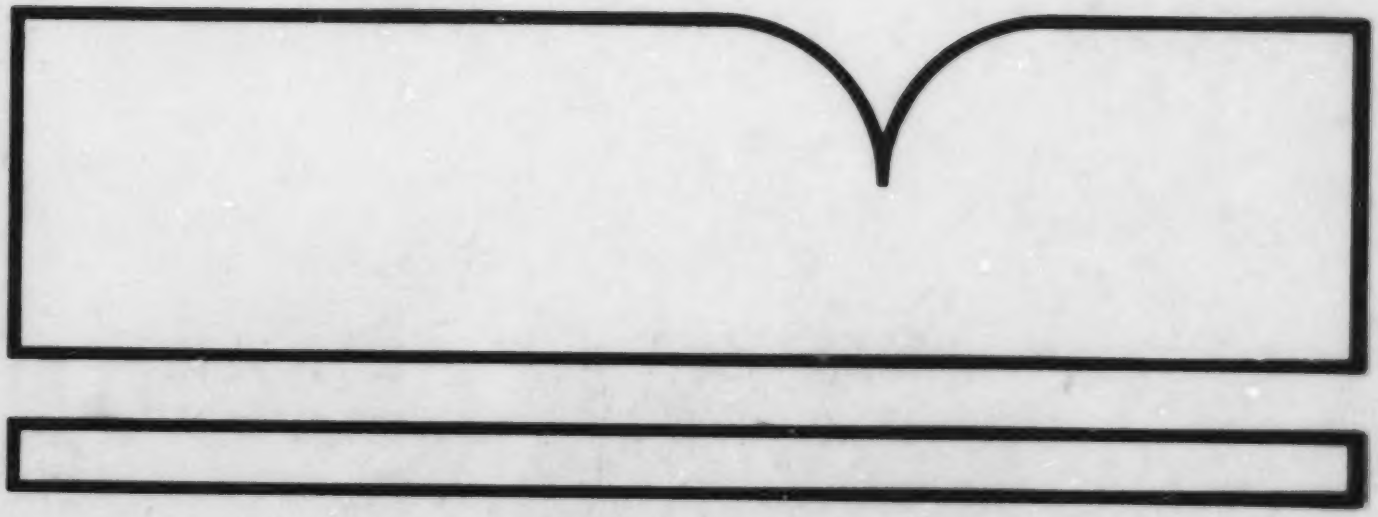

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REDUCTION OF TOXICITY TO AQUATIC ORGANISMS BY INDUSTRIAL WASTEWATER TREATMENT

by

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16. ABSTRACT

The specific goal of this research was to conduct 24-hour static acute bioassays with "untreated" influent and "treated" effluent using fathead minnows (Pimephales promelas) and water flea (Daphnia magna) to biologically evaluate the effectiveness of industrial wastewater facilities in reducing effluent toxicity to aquatic organisms. Of primary interest to the EPA was an evaluation of the capacity of the wastewater treatment facilities of the pesticide industry for reducing toxicity. To accomplish the stated goal, on-site 24-hour static acute toxicity tests were performed during ten consecutive workdays at seven industrial sites. Five of the test sites are defined as pesticide manufacturers, while the remaining sites consisted of an organo-chemical manufacturer and a bleached-kraft paper mill. The effectiveness of the treatment plants was determined by performing static acute toxicity tests with the fathead minnow (Pimephales promelas) and the water flea (Daphnia magna) on samples of the wastewater collected before and after treatment. Results of the studies are expressed in terms of both median lethal concentrations (LC50's) as % effluent and lethal units.

The data from these studies indicate that the wastewater treatment plants provided an average efficiency of 98% in reducing the toxicity of "untreated" wastewaters. Neither species tested proved to be a more sensitive indicator of toxicity, though a larger data base is required to make valid appraisal.

Of interest was the observation that while some wastewater treatment facilities provide good efficiency (98+%) in reducing toxicity, the resulting effluent still represented a relatively high number of lethal units. This was a result of the fact that the "untreated" influent entering the waste treatment system contained a very high level of lethal units and a subsequent 98% reduction of that level still resulted in a toxic wastewater.

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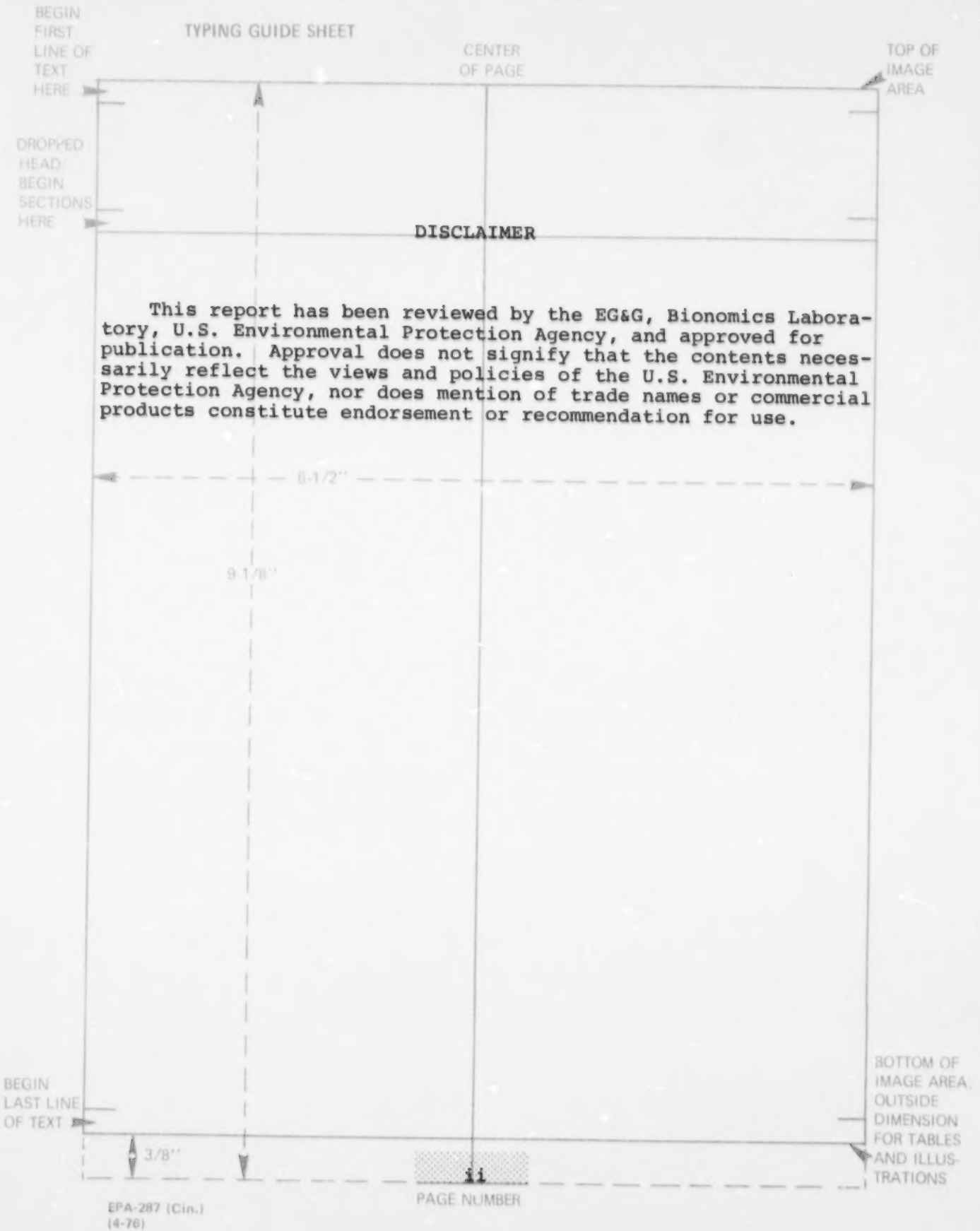

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EXECUTIVE SUMMARY

On-site, 24-hour static acute toxicity tests were performed during 10 consecutive work days at 7 industrial sites to determine the effectiveness of the respective wastewater treatment facilities in reducing toxicity of the discharges to aquatic organisms. Five of the test sites are defined as pesticide manufacturers, while the remaining sites consisted on an organochemical manufacturer and a bleached-kraft paper mill. The effectiveness of the treatment plants was determined by performing static acute toxicity tests with the fathead minnow (Pimephales promelas) and the water flea (Daphnia magna), on samples of the wastewater collected before and after treatment. Results of the studies are expressed in terms of both median lethal concentrations (LC50's) as % effluent and lethal units.

The data from these studies indicate that the wastewater treatment plants provided an average efficiency of 98% in reducing the toxicity of untreated wastewaters. Neither species tested proved to be a more sensitive indicator of toxicity, though a larger data base is required to make valid appraisal.

Of interest was the observation that while some wastewater treatment facilities provide good efficiency (98+%) in reducing toxicity, the resulting effluent still represented a relatively high number of lethal units. This was a result of the fact that the untreated influent entering the waste treatment system contained a very high level of lethal units and a subsequent 98% reduction of that level still resulted in a toxic wastewater.

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Additionally, the authors would also like to express their sincere appreciation to each of the companies participating in this study for the extensive time and effort they devoted in supporting the requirements of Bionomics' mobile bioassay unit and laboratory personnel.

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SECTION 1

INTRODUCTION

In 1977, the United States Environmental Protection Agency was charged by Congress under the Federal Water Pollution Control Act Amendments (PL-95-217) to develop a program which would protect aquatic life by eliminating the discharge into the nation's waterways of toxic pollutants in toxic amounts. Under contract to the EPA (#68-03-2631), EG&G, Bionomics' personnel performed on-site toxicity testing at seven industrial wastewater treatment facilities throughout the United States. The data generated from these studies will aid the Agency in developing effluent discharge guidelines related to the toxicity to aquatic organisms of industrial wastewater streams.

The specific goal of this research was to conduct 24-hour static acute bioassays with "untreated" influent and treated effluent using fathead minnows (Pimephales promelas) and water flea (Daphnia magna) to biologically evaluate the effectiveness of industrial wastewater facilities in reducing effluent toxicity to aquatic organisms.

To accomplish this task, it was necessary to evaluate the toxicity of the wastewaters before and after biological and/or chemical treatment procedures. Results of the acute toxicity studies are expressed in both the conventional format as median lethal concentrations (LC50's) and as lethal units, a concept which is similar to that of toxic units as described by Esvelt, Kaufman and Selleck (1971). The lethal unit concept as it applies to effluents, is the concentration of the effluent divided by the 24-hour LC50. This unitless expression of toxicity may be ideally suited for use with wastewater discharges of diversified effluents and can be incorporated into the regulatory process of controlling and limiting toxic discharges.

Of primary interest to the EPA was an evaluation of the capacity of the wastewater treatment facilities of the pesticide industry for reducing toxicity. The Agency's rational in selecting this industry was based on the premise that discharges from pesticide manufacturers might be expected to be relatively toxic and perhaps not readily degraded even during biological treatment. The industries which participated in this study were Monsanto Company, Muscatine, Iowa; Mobay Chemical Company, Kansas City, Missouri; Monsanto Company, Luling, Louisiana; and Diamond

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Shamrock Corporation, Green Bayou, Texas. Most chemical manufacturing complexes are not limited to the production of pesticides alone. These sites were selected because they had segregated pesticide wastewater treatment facilities which do not (for the most part) receive wastewater from other manufacturing processes and because the majority of the treatment systems were candidate best available technology economically achievable (BAT) systems or portions thereof. In addition to the above 4 pesticide manufacturers, studies were conducted with a complex organochemical effluent at Union Carbide Corporation, South Charleston, West Virginia, a complex organo-pesticide effluent at E.I. duPont Nemours & Company, Inc., LaPorte, Texas and a bleached-kraft paper mill effluent from International Paper Company, Georgetown, South Carolina. Table 1 presents the sites in order visited along with dates and other pertinent information.

Contained in this report is a description of the test procedures and results of the biological and chemical findings at each site.

SECTION 2

MATERIALS AND METHODS

On-site testing was carried out at each site in one of EG&G, Bionomics' Mobile Aquatic Toxicology Laboratories. Unless otherwise stated, procedures used in conducting the on-site tests followed "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (EPA, 1975). All raw data generated from these studies are permanently stored in the archives at EG&G, Bionomics, Wareham, Massachusetts.

Dilution Water

The standard diluent and control water used throughout this contract was a constant quality reconstituted "hard water" prepared according to EPA (1975) methodology. A potable water supply was used to prepare the reconstituted water at each site. The potable water was first filtered through an activated carbon cylinder and then through mixed bed deionizers which resulted in dechlorinated deionized water with a conductivity of less than 1 micromho per centimeter (μmho/cm). The specified amounts of the reagent-grade chemicals were then added to this water to prepare batches of 1000 liters (ℓ) each of the reconstituted diluent.

Each batch of reconstituted water was then mixed for at least 1 hour in order to allow for complete dissolution of the chemical salts. Mixing was accomplished by intensive aeration using a Zodar^{R} aeration system. The resulting water was characterized by having a total hardness of 160-180 mg/ℓ as calcium carbonate ($CaCO_3$), a total alkalinity of 110-120 mg/ℓ as $CaCO_3$, a pH of 7.6-8.0, and a specific conductivity of 400-600 μmhos/cm. Prior to use, the temperature of the reconstituted water was adjusted, as necessary, to 22^{o}C by use of either a thermostatically controlled Thermoquartz^{R} (MT3-905), 9000 watt stainless steel immersion heater or 1 H.P. Fridgid^{R} Unit chiller (epoxy coated) equipped with a self-contained thermostat.

Wastewater Sampling

The specific design of the wastewater treatment plants varied from one site to the next depending on the nature and composition of the wastewater being treated. Wastewater samples, which were generally representative of the major contributory

pesticide wastewater stream entering the treatment plant ("untreated" influent) and the final discharge stream from the wastewater treatment plant ("treated" effluent), were collected at each site.

Depending on the site, 1-2 "untreated" influent wastewater streams were tested. In certain instances, direct access to an incoming wastewater stream was not possible until after it had already entered some portion of the treatment system (e.g., grit basin or pH neutralization basin). At these sites the influent samples were obtained immediately prior to the next step in the treatment process. The complexity of some wastewater treatment plants (especially E.I. DuPont, LaPorte, Texas) made it impossible to test influent samples which were wholly representative of the total pesticide wastewater load entering the treatment system. This was a result of multiple wastewater streams entering the treatment plant at different stages in the treatment process.

"Treated" effluent samples were obtained at sampling points located immediately after the last step in the treatment process. Since many of the sites have multiple wastewater treatment systems which are utilized for treating other manufacturing processes, the "treated" effluent tested in these studies represented only the wastewater discharged directly from the pesticide wastewater treatment system. These samples were collected prior to dilution with other process wastewater streams or non-contact cooling waters and therefore are not representative of the company's total discharge entering the receiving stream.

Test Organisms

Fathead minnow--Fathead minnows (Pimephales promelas) used in these studies were raised from eggs in the culture facilities of EG&G, Bionomics. These fish were cultured in water which was a mixture of Bionomics' well water and Town of Wareham non-chlorinated, non-treated well water. The culture water is characterized by having a range of total hardness and total alkalinity of 25-39 mg/ℓ and 20-39 mg/ℓ as $CaCO_3$, respectively, a pH of 6.7-7.2, a specific conductance of 70-145 µmhos/cm and a temperature of 25 $\pm$ 2°C.

Fish, 21-39 millimeters (mm) total length, 0.1-0.4 grams (g) wet weight were acclimated to 22°C well water over a 24-hour period. The aforementioned measurements for each group of fish are based on 30 individuals. The fish were then held at this temperature for a minimum of 5 days prior to shipment by air to each site. Upon arrival at a test site, the fish (1,500-5,000) were transferred to a 600-liter insulated fiberglass holding tank maintained inside the mobile laboratory. This tank was fitted with a standpipe drain which maintained a tank volume of approximately 500 liters. Water flowing into this tank was from the

potable water supply which had first been dechlorinated (activated carbon) and adjusted to 22 ± 1°C. During the first 18-24 hours of acclimation, the flow rate entering the holding tank was increased in stages from 0.2 ℓ/minute to 4 ℓ/minute. A flow rate of 4 ℓ/minute was then maintained for the duration of the study and provided 11.5 volume replacements per day.

Acclimation of fathead minnows to the dilution water (reconstituted water) was performed twice daily, once in the morning and once in the afternoon. At each interval, 100-150 fish were transferred with approximately 15 liters of dechlorinated potable water to one of two 125-liter stainless steel acclimation tanks. Reconstituted water (22°C) was then introduced to each tank at a flow rate of 250 mℓ/minute. The tanks were maintained at a constant volume of 90 liters.

The fish were acclimated to reconstituted water for a 24-hour period prior to use in a test. The holding tank was then drained and the acclimation process repeated with a new group of fish.

Fathead minnows in the main holding tank were fed a dry commercial fish food once a day following the afternoon transfer of fish to the stainless steel holding tank. Fish were not fed during the 24-hour acclimation period in the dilution water nor during the performance of the tests. Cumulative mortality during the 30-day holding periods prior to shipment of the fish to each site and during the holding and acclimation periods on-site did not exceed 1%.

Water flea--The water flea (Daphnia magna) used in this study were obtained from laboratory stocks cultured at the Wareham laboratory. The culture water used for the daphnids was reconstituted deionized well water characterized by a total hardness of 160-190 mg/ℓ as $CaCO_3$, total alkalinity of 110-130 mg/ℓ as $CaCO_3$, a pH of 7.8-8.2, a specific conductivity of 500-700 µmhos/cm, a temperature of 21-23°C, and a dissolved oxygen concentration greater than 60% of saturation (5.2-5.4 mg/ℓ). The daphnid cultures were fed a diet of unicellular green algae and commercial fish feed.

Large gravid females were selected from the laboratory cultures and shipped by air in approximately ten liters of culture water to each testing location. Upon arrival at each test site, the cultures were transferred to three 60-ℓ glass aquaria. Reconstituted dilution water was used to supplement the original culture water to yield a total volume of 40 ℓ/aquarium. Water in each aquarium was then gently aerated. The cultures were maintained under static conditions at 22 ± 1°C. D. magna were fed daily a commercial fish feed which had been mixed with reconstituted water in a glass blender cup.

The daphnids used in the on-site testing were generally less than twenty-four hours old. The procedure for obtaining the test organisms was to first transfer an adequate number of gravid adult daphnids from the cultures to a 2-ℓ battery jar containing 1.7 ℓ of reconstituted water. On the following day, the first instar daphnids released from the adults were removed and randomly distributed to the test chambers for testing. On those occasions when the isolated adults failed to release a sufficient number of young, the amount required for testing was supplemented by collecting first instar daphnids from the main culture aquaria.

On-Site Test Procedures

General-- Twenty-four hour static acute toxicity tests were performed daily on samples of "untreated" influent and "treated" effluent at each of the study sites (Mobay Chemical, only "treated" effluent) over ten consecutive test days. Fathead minnow tests were conducted on wastewater samples collected at two sampling periods each day while daphnid tests were performed on alternating days beginning with test day 1. At 4 of the sites, Salmonella Bacterial Mutagenesis Assays (Ames Test) were performed on samples of the "treated" effluent.

Grab samples of each wastewater (influent and effluent) were obtained twice a day (9:00 a.m./3:00 p.m.) for ten consecutive days to coincide with the test regimentation. A sample of each wastewater was taken prior to the first day of testing in order that preliminary, range-finding tests could be conducted with each test species. At certain sites, personnel from the "host" company collected duplicate or "split" samples of the wastewater with the laboratory personnel from EG&G, Bionomics.

Each grab sample was collected in a separate polyethylene bucket at each sampling location and transferred to individual 53-ℓ Nalgene^{R} polyethylene carboys. The total sample volume ranged from 20-50 ℓ and was dependent on the toxicity of each wastewater. Upon delivery to the mobile laboratory facility, a 500 mℓ subsample was drawn from each wastewater prior to temperature modification in order to conduct limited wastewater characterization. The wastewater samples were then heated or chilled as necessary to 22 + 2°C. This was accomplished by pumping each wastewater sample through individual stainless steel coils which were immersed in the main diluent tank. Once the desired test temperature (22°C) of each wastewater was attained, the samples were immediately used to initiate the respective toxicity tests scheduled for that day.

A characterization of the chemical and physical parameters of the dilution water and "treated" and "untreated" effluent wastewaters was performed daily at each site. On each test day, samples of reconstituted dilution water were analyzed for total hardness, total alkalinity, specific conductance, pH, temperature

and total residual chlorine. Similarly, aliquots of each wastewater sample collected during each ten day study were analyzed for dissolved oxygen concentration (DO), pH, specific conductance, temperature and total residual chlorine. These measurements were made on samples of the wastewaters brought back to the mobile laboratory, but prior to temperature adjustment.

During each test, the pH, dissolved oxygen concentration and temperature were measured in the control, high, middle and low test concentrations prior to the addition of the test organisms and at the termination of the test. Specific conductance was monitored similarly but only at the initiation of testing.

Total hardness and alkalinity measurements were performed according to Standard Methods (APHA et al., 1975, pg. 179 and 53, respectively). Specific conductance was measured with a Model #33 YSI conductivity meter, pH with a Model #175 Instrumentation Laboratory pH meter and combination electrode and dissolved oxygen with a YSI Model #57 DO meter. Total residual chlorine measurements were performed on a Fisher and Porter Model #17T1010 amperometric titrator according to either the "forward" titration procedure (APHA et al., 1975, p. 112) or the "backward" titration method (Fisher and Porter Bulletin 17T1010, pg. 7). Temperature maintained at 22°C was monitored with either a hand held mercury column thermometer and/or YSI Model #80A recorder. All measurements were performed using the appropriate metering devices previously described. Each metering device was calibrated once a day for ten consecutive test days.

Fathead Minnow Tests

Static acute toxicity tests with fathead minnows were conducted at a minimum of 5 concentrations (0.65 dilution factor between adjacent concentrations) plus control (diluent water). Test vessels were 19.6-ℓ, all-glass aquaria with a test volume of 15 ℓ. Each wastewater sample was transferred by pump or glass graduated cylinder from the sample carboys (temperature adjusted) to the appropriate test chambers. Volumes in excess of 2 ℓ were generally introduced into the aquaria with a pump. The sides of the aquaria were calibrated as to the exact volume of wastewater required for the respective concentrations. Initial calibration of a marked aquarium was performed with a Type A graduated cylinder. Dilution water was then added by pump to each aquarium to bring the total volume to 15 ℓ, indicated by an additional permanent mark on the side of each aquarium.

After all aquaria for each wastewater test were filled, the water quality parameters previously described were measured and recorded. Fathead minnows were then transferred by net (usually two at a time) from the dilution water acclimation tank to the test aquaria and the test was initiated. A total of 10 fish per concentration was tested.

After twenty-four hours of exposure, observations of mortality and physical/behavioral abnormalities were made and recorded for fish in each test aquarium. The water quality parameters (pH, DO, temperature) were measured and recorded prior to test termination. The test aquaria for each wastewater were then immediately drained, brushed down, and rinsed thoroughly in order that a new test could be set up immediately in the same respective aquaria.

Daphnia magna Tests

Daphnia magna acutes with each wastewater were initiated using a minimum of five test concentrations plus a control in triplicate. The dilution factor for each wastewater concentration was also 0.65.

The diluent water used in these studies was the same as that described previously. Each test concentration was prepared by measuring out the wastewater in a Type A 500-mℓ graduated cylinder. Dilution water was then transferred to a 1-ℓ beaker, mixed on a magnetic stirrer and quantitatively divided among 3 replicate 250-mℓ beakers. The A replicate of each concentration received 200 mℓ of test solution while the remaining two replicates received 150 mℓ each. The additional volume in the A replicate was necessary to accomodate the meter probes used for daily water chemistry measurements.

As was done in the fish acutes, water quality parameters were monitored at the beginning of the study prior to addition of the test organisms and again at the end of the test. Five daphnids were randomly assigned to each test vessel within 30 minutes after the introduction of each wastewater sample, a total of 15 daphnids per concentration. All tests were carried out at 22 $\pm$ 2°C.

Ames Test

Salmonella Mutagenicity Tests (Ames Test) were carried out on samples of "treated" wastewater from the four industrial wastewater treatment plants indicated in Table 1. The tests were conducted at EG&G, Mason Research Institute, Rockville, Maryland. The specific protocol followed is presented in the Appendix. At each participating test site, a 100-mℓ subsample of the 9:00 a.m. "treated" wastewater (effluent) sample was collected in a clean 235-mℓ glass bottle, sealed with a Teflon^{R}-lined cap and frozen. Sampling occurred on the first day of testing and then on an alternate day basis. At the end of the ten consecutive days of testing, the five samples were packed with dry ice and shipped, by air, to the Mason Research Institute, where the samples were thawed out and diluted to the appropriate volume prior to testing.

Statistics

Mortality data derived from each definitive test were used to calculate a median lethal concentration (LC50) and its 95% confidence limit utilizing the moving average angle method (Harris, 1959). The data are presented as both LC50 values (percent wastewater) and lethal units (100/LC50). The LC50 is the calculated nominal concentration of the wastewater in diluent water which produces mortality of 50% of the test animal population at the stated time of exposure; i.e., 24-hour LC50. In those instances where LC50 values could not be calculated due to <50% mortality in 100% wastewater, the lethal units have been derived through graphical interpolation of the data on log x probit paper and provide the most probable toxicity concentration (Esvalt, et al., 1971). All data are presented after rounding to two significant figures.

SECTION 3

RESULTS

Union Carbide, South Charleston, West Virginia

On-site toxicity tests with fathead minnow and Daphnia magna were conducted at the South Charleston Waste Treatment Works from September 27 - October 15, 1978. The wastewater treatment plant treats both industrial and domestic wastewaters which are discharged to the Kanawha River through a common discharge pipe. The industrial portion of the wastewater is derived from the Union Carbide complex in South Charleston.

Due to its nature, the industrial wastewater stream enters the plant as a separate influent stream and remains segregated from the domestic wastewaters throughout the treatment process. Overflow from the secondary clarifiers of the industrial treatment system combines with the municipal effluent to form the primary discharge to the river. A flow diagram of the industrial wastewater treatment system with identification of the sample points is presented in Figure 1.

Tests with "untreated" influent and "treated" effluent were performed according to the scope of work described previously. Results of the acute toxicity studies are presented in Table 2. These data indicate that the "untreated" influent was relatively toxic to both test species while no mortality was observed in either test population exposed to the "treated" effluent throughout the ten day testing period. The wastewater treatment process provided a 100% reduction in lethal units (10 units) for fathead minnow and a 100% reduction (12 units) for daphnids.

Measured water quality parameters of the major test waters, i.e., influent wastewater (untreated), effluent (treated) and diluent water (reconstituted) are presented in Table 3. The data, compiled during the ten consecutive days of on-site testing are tabularized as the means and range for each parameter. As would be expected, the measured parameters for the "untreated" influent varied over a greater range than were observed for the "treated" effluent.

A summary of the water quality parameters measured in exposure aquaria during the fish and daphnid toxicity tests with each wastewater are presented in Tables 4 and 5. It should be noted

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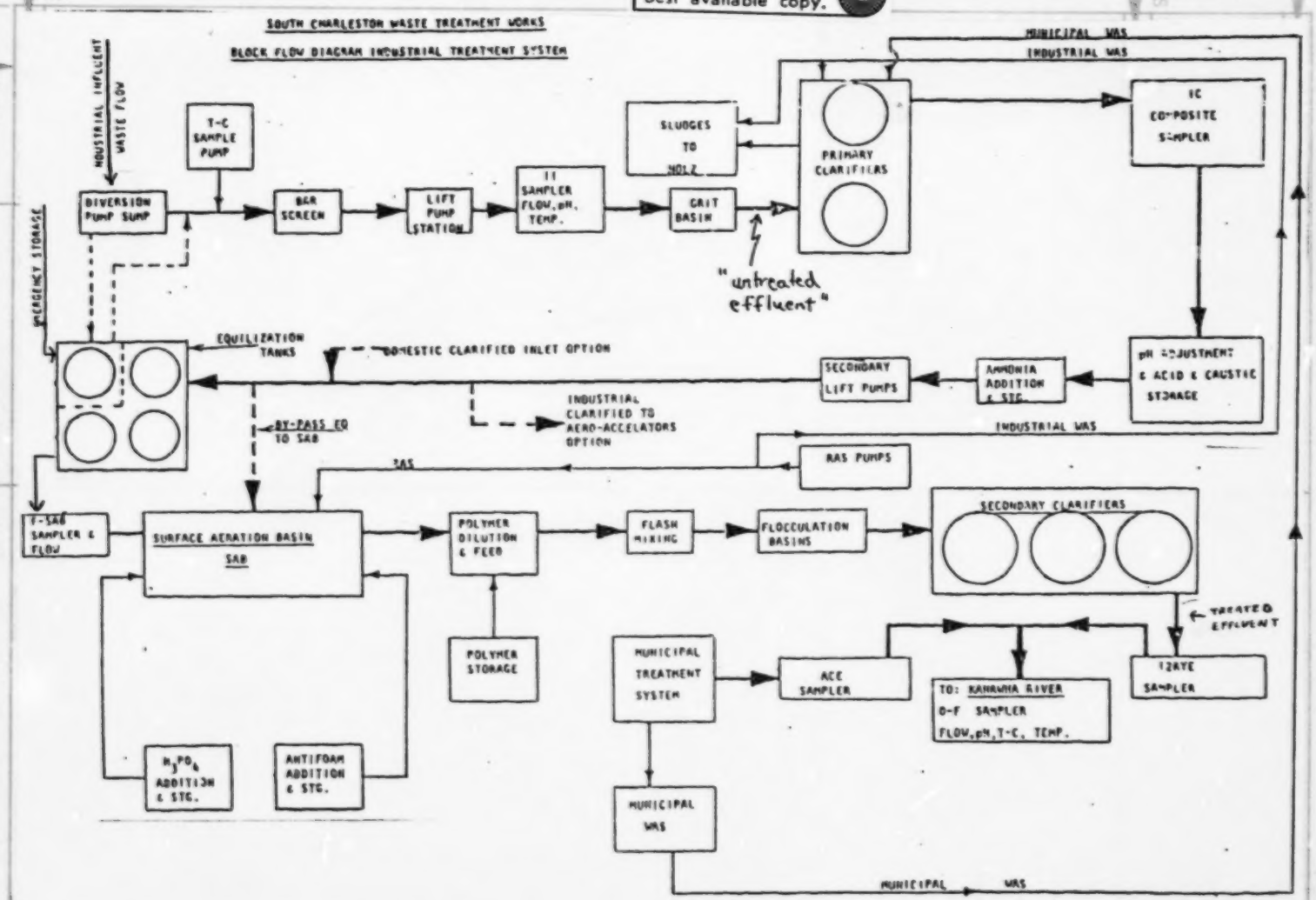

FIGURE 1. FLOW DIAGRAM FOR WASTEWATER TREATMENT PLANT AT SOUTH CHARLESTON, WEST VIRGINIA (UNION CARBIDE EFFLUENT).

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that the high, middle and low test concentrations for the "untreated" influent were changed during the ten days of testing as a result of the observed toxicity of this wastewater which guided the selection of wastewater concentrations to be tested. The concentration range used for any given test was based on the test results from toxicity data generated from the previous day. An attempt was made to try and obtain an effect/no effect level for each test. The data presented in Tables 4 and 5 indicate that the dissolved oxygen concentration and pH varied minimally between treated effluent concentrations for either species. A wider range of values were observed during the testing of the "untreated" influent. These data also indicate that the specific conductance varied directly with the influent/effluent concentration.

Samples for the Salmonella mutagenicity tests (Ames test) were not obtained at this site.

Monsanto, Muscatine, Iowa

On-site toxicity tests with fathead minnow and Daphnia magna were conducted at the Monsanto Company, Muscatine, Iowa from October 17-29, 1978. The wastewater treatment facility consists of two separate treatment plants. One plant (ABS Plastics) handles wastewaters from the production of intermediate chemicals for the production of plastics, while the second plant treats wastewaters from the agricultural chemicals facility. Treated wastewaters from the treatment plants combine with large amounts of non-contact cooling water from the 001 discharge entering the Mississippi River. For the purpose of this contract, only the treated effluent from the Agricultural Chemicals treatment plant was tested. "Untreated" influent was obtained prior to pH adjustment while "treated" effluent was obtained from the overflow of the primary clarifiers. A flow diagram of the wastewater treatment process is presented in Figure 2.

Results of the acute toxicity studies with the "untreated" influent and "treated" effluent are presented in Table 6. As expected, the "untreated" influent was quite toxic to both test species. The mean number of lethal units (LC50's) contained in the influent were 47(2.3%) and 41(2.9%), respectively, for fathead minnows and daphnids. Results of the "treated" effluent studies indicate a 97% reduction in toxicity after treatment for both fathead minnows (1.5 lethal units, 68%) and water flea (1.3 lethal units, 75%).

The measured water quality parameters of the "untreated" influent, "treated" effluent and reconstituted diluent water compiled during the ten consecutive days of on-site testing are presented in Table 7. The pH and conductivity of the influent exhibited considerable variation from one sample to the next, while for the "treated" effluent all of the measured parameters remained

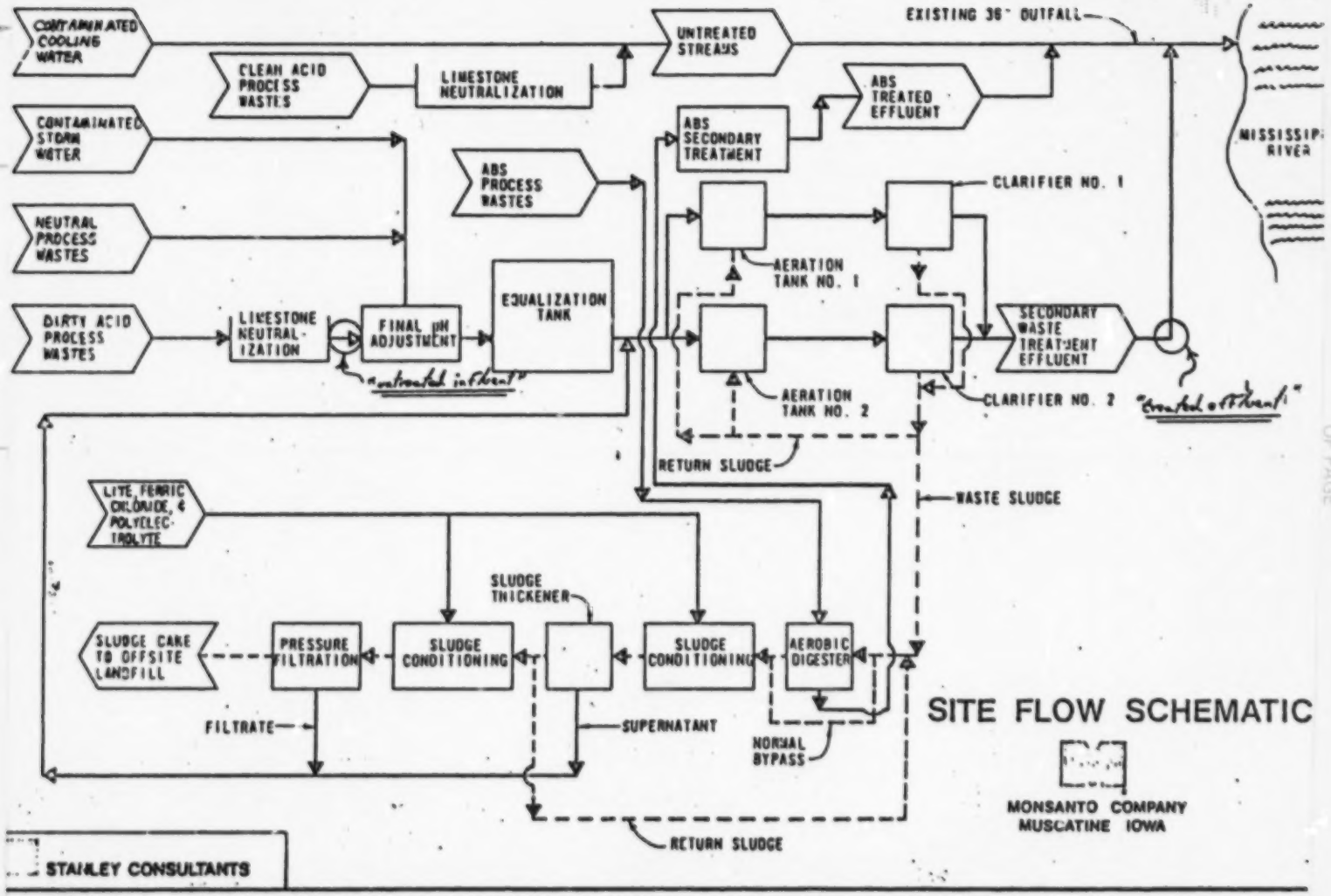

FIGURE 2. FLOW DIAGRAM FOR WASTE TREATMENT FACILITY AT MONSANTO, MUSCATINE, IOWA.

relatively constant.

A summary of the water quality parameters measured in the exposure aquaria during the fathead minnow and daphnid toxicity tests with each wastewater are presented in Tables 8 and 9. Variations in the pH and specific conductance of the "untreated" influent were directly correlated with the toxicity of the wastewater. Consequently, the data presented for the high, middle and low test concentrations represent ranges for the respective dose levels which varied accordingly from day to day.

The water quality parameters monitored during the tests with both wastewaters remained relatively constant within the same set of concentrations. This probably is a result of the moderating effects of the dilution water which was used in proportionally greater volumes in setting up the "untreated" influent tests. Even at these low test concentrations a significant oxygen demand was present and may have contributed to the observed mortalities.

The results of the Salmonella mutagenicity assay of the "treated" effluent sampled on days 1, 3, 5, 7 and 9 indicate that there was no consistent significant increase in the reversion index of any of the tester strains with or without metabolic activation.

Mobay Chemical, Kansas City, Missouri

On-site toxicity tests were conducted at the Mobay Chemical Company, Kansas City, Missouri during the period November 3-7, 1978. On November 2, EG&G, Bionomics was informed that the Environmental Protection Agency had accepted Mobay's request to omit the tests with the "untreated" influent and consequently the on-site tests were limited only to the "treated" effluent. The "treated" effluent was obtained from the biological effluent flume; a concrete raceway where the outfall from two clarifiers are channeled. After leaving the flume, these wastewaters are combined with the discharge from the Kansas City Electric Generating Plant, which subsequently flows into the Blue River. A flow diagram of the wastewater treatment plant at Mobay was not provided.

Results of the acute toxicity studies with the "treated" effluent are presented in Table 10. These data indicate that the mean acute LC50 value for fathead minnow and daphnids exposed to the "treated" effluent were 28% (3.8 lethal units) and 23% (4.4 lethal units), respectively. The efficiency (in terms of % reduction of lethal units) of the wastewater treatment facility could not be determined as a result of the omission of the tests with "untreated" influent.

The measured water quality parameters of the "treated" effluent and reconstituted diluent water, monitored during the

on-site testing, are presented in Table 11. These data suggest that no significant variation was observed throughout the testing period.

A summary of water quality parameters measured in the high, middle and low exposure concentrations and control during the fathead minnow and daphnid toxicity tests with the "treated" effluent are presented in Table 12. These data indicate little variation in the initial dissolved oxygen concentration or pH for each test throughout the testing period. Specific conductance varied directly with the concentration of effluent. Dissolved oxygen concentrations observed at the termination of the fathead minnow tests, though significantly reduced from the 0-hour reading, were generally adequate to support aquatic life.

The results of the Ames Test performed on the "treated" effluent water samples from Mobay Chemical, obtained on test day 1, 3, 5, 7 and 9 indicate that none of the samples caused a significant increase in the reversion index of any of the test strains with or without metabolic activation.

Monsanto, Luling, Louisiana

On-site toxicity tests with fathead minnow and Daphnia magna were conducted at Monsanto, Luling, Louisiana during the period December 1-17, 1978. The Luling complex manufactures a number of chemical products in addition to the glyphosate product Roundup^{R}. The wastewater treatment plant received the wastewater from the Roundup facility as well as from the other manufacturing processes. Therefore, the samples of "untreated" influent and "treated" effluent were representative of the combined processes of the Luling complex. Samples of the influent were obtained from the overflow of the collection basin after acid-base neutralization, while the "treated" effluent was obtained from the discharge weir of the aeration lagoon. A flow diagram of the wastewater treatment system is presented in Figure 3.

Table 13 presents the results of the 24-hour static acute toxicity tests with "untreated" influent and "treated" effluent. The "untreated" influent exhibited a mean of 74 lethal units (LC50, 1.5%) for fathead minnows and 88 lethal units (LC50, 1.3%) for the water flea. After treatment, the mean number of lethal units for fatheads and daphnids were 6 (LC50, 18%) and 3 (LC50, 33%), respectively. Although these values indicate that the "treated" effluent is still moderately toxic to both test species they do represent 92% (fathead minnow) and 96% (daphnid) reduction in toxic units by the wastewater treatment facility.

Water quality parameters monitored on the influent, effluent and dilution water during the testing period are presented in Table 14. A summary of the water quality parameters measured in the exposure aquaria during the fathead minnow and daphnid toxic-

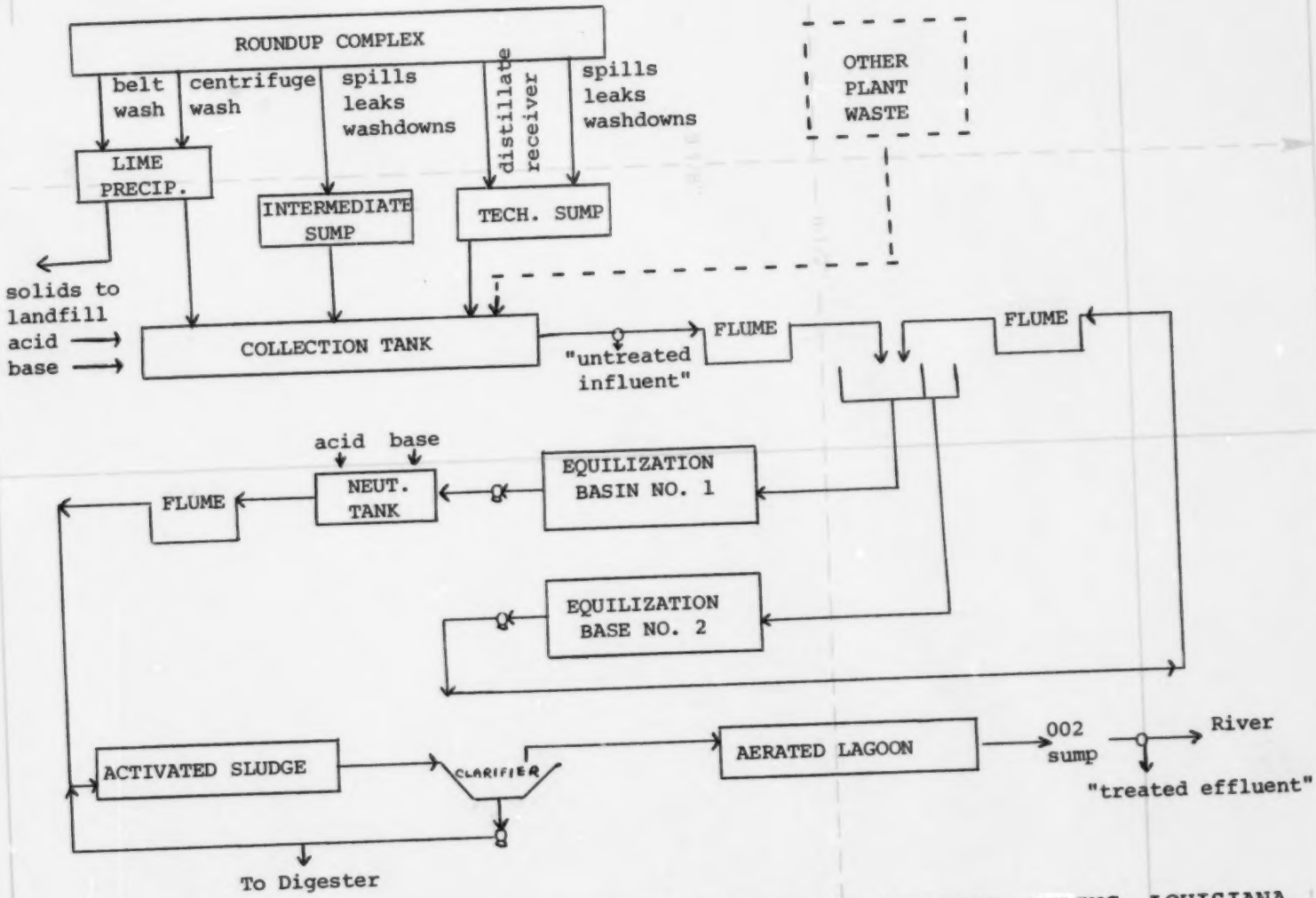

FIGURE 3. FLOW DIAGRAM FOR WASTE TREATMENT FACILITY AT MONSANTO, LULING, LOUISIANA.

ity tests with each wastewater is presented in Tables 15 and 16. The high, middle and low test concentrations for each wastewater were varied on a day to day basis, when necessary, in an attempt to obtain effect/no effect tests. The data presented in Tables 15 and 16 indicate that the initial (0-hour) dissolved oxygen concentration and pH varied minimally in both wastewaters and that the specific conductance varied directly with the influent/effluent concentration. The measurements made at the end of the fathead minnow tests with "treated" effluent indicate DO concentrations in the high concentration were at times marginal.

The results of the Ames Test performed on the "treated" effluent water samples collected on test day 1, 3, 6, 7 and 9 indicate a weak but consistent increase in the reversion index of tester strain TA 1535. The dose related increase was present both with and without metabolic activation but was most pronounced in the presence of rat liver microsomes.

A confirmation study of the His^+ phenotype of "revertant" colonies of strain TA1535 at the highest dose level indicated that 91% of the colonies were true revertants. This result supports the conclusion that the observed increase in reversion index of TA1535 is real and not an artifact of the experiment.

Diamond-Shamrock, Green Bayou, Texas

On-site toxicity tests with fathead minnow and Daphnia magna were conducted at Diamond-Shamrock's Green Bayou, Texas plant during the period January 3-22, 1979. The Green Bayou complex manufactures a number of chemical products in addition to its major product Dakonyl^R, a fungicide. Samples of the "untreated" influent were obtained from the inflow pipe prior to introduction into the primary settling basin. Samples of "treated" effluent were obtained from the discharge raceway of the aeration lagoon. A flow diagram of the wastewater treatment facility was not provided.

Results of the acute toxicity studies with the "untreated" influent and "treated" effluent are presented in Table 17. The "untreated" influent was considerably more toxic to fathead minnow (mean = 360 lethal units, LC50, 3.3%) than to the water flea (mean = 130 lethal units, LC50, 2.1%). After treatment, the toxicity of the wastewater was reduced to 1.2 lethal units (LC50, 84%) for fathead minnows and 2.6 lethal units (LC50, 38%) for Daphnia magna. This is a reduction in lethal units of 99.7% and 98% for the fish and daphnids. In comparing the mean LC50's to the mean lethal units for the "untreated" influent tests, it would appear that the values are incorrect in that the mean LC50 divided into 100 does not equal the mean lethal units. Results of several of the acute tests indicate that at times the "untreated" influent was extremely toxic. When these LC50 values are converted to lethal units, they tend to skew the mean.

Water quality parameters monitored for the two wastewaters and dilution water are presented in Table 18. Table 19 and 20 present a summary of the water quality parameters measured in the high, middle and low exposure aquaria including the control during toxicity tests with fish and daphnids. As in previous tests with other wastewaters, the dissolved oxygen concentrations and specific conductance varied directly with the wastewater concentration. DO levels were again marginal in the high concentrations of the fish tests with both the influent and effluent.

The results of the Ames Test performed on the "treated" effluent water samples from the Diamond-Shamrock plant in Green Bayou, Texas on days 1, 3, 5, 7 and 9 indicate that none of these samples caused a significant increase in the reversion index of any of the tester strains with or without metabolic activation.

E.I. duPont de Nemours, LaPorte, Texas

On-site toxicity tests with fathead minnows and Daphnia magna were conducted at duPont's LaPorte, Texas plant during the period February 9-24, 1979. The LaPorte complex manufactures a number of chemical products and pesticides which includes Maneb and Methomyl. Samples of two "treated" influents were collected at this site. One influent wastewater (Stream #1) was collected from the process wastewater ditch after pH adjustment. This wastewater stream was comprised of several pesticide/non-pesticide unit effluents and constituted the main flow into the treatment system. The second influent (Stream #2) was collected from the Maneb/Methomyl wastewater stream prior to chemical oxidation with chlorine. The "treated" effluent was obtained from a spigot located at the outfall pond #101. The "treated" effluent combines with the non-contact wastewater from outfall #201 prior to discharge into San Jacinto Bay. A flow diagram of the wastewater treatment facility was provided to EG&G, Bionomics labelled confidential and therefore is not included in this report.

Results of the acute toxicity studies with fathead minnow and daphnids to two "treated" influents and the "treated" effluent are presented in Tables 21 and 22. A complete regimentation in testing of the "treated" influent collected from the Maneb/Methomyl production stream could not be accomplished due to a malfunction in the plant equipment and subsequent process shutdown on test day #4. The data from the acute toxicity studies indicate that the mean number of lethal units (LC50) for fathead minnow exposed to the "treated" influent (pesticide/non-pesticide stream #1) was 4.5 lethal units (27%), while the partial testing of the "treated" influent (Maneb/Methomyl prior to chemical oxidation, stream #2) resulted in an acute toxicity of 140 lethal units (0.75%). The mean for fathead minnow exposed to the "treated" effluent was 2.4 lethal units (42%). This indicates that the wastewater treatment process provided 47% and 95% reduc-

tion in toxicity (2.2 and 134.6 lethal units) to the two "treated" influents, respectively.

The mean number of lethal units (LC50) for daphnids exposed to the two "treated" influents (pesticide/non-pesticide and Maneb/Methomyl) and the "treated" effluent were 4.8(28%), 770 (0.14%) and 35(4.4%), respectively. These data would indicate that the wastewater treatment process provided a 95% reduction in toxicity to daphnids exposed to the Stream #2 influent (Maneb Methomyl), but that no reduction in toxicity was observed when compared to the results of the Stream #1 influent. Due to the physical limitations inside the mobile laboratory, all of the incoming wastewater streams entering the treatment plant could not be tested. Therefore, an accurate interpretation of the efficiency of the duPont wastewater treatment facility cannot be made.

The measured water quality parameters of the "treated" influent (Stream #1), the "treated effluent and reconstituted diluent water, compiled during the ten consecutive days of on-site testing, are presented in Table 23. These data suggest that the "treated" influent was more variable with respect to all measured parameters than was the "treated" effluent.

A summary of the water quality parameters measured in the exposure aquaria during the fathead minnow and daphnid toxicity tests with each wastewater are presented in Tables 24-27. These data indicate that the greatest variation in any measured parameter was the pH observations for the "treated" influent from the pesticide/non-pesticide stream #1. The dissolved oxygen concentrations observed after 24 hours in all three test waters decreased in direct proportion to the test concentration while the specific conductance increased in direct relation to the test concentrations.

Samples of the Salmonella/mammalian-microsome mutagenicity test (Ames Test) were omitted at this test site at the request of the Project Officer.

International Paper, Georgetown, South Carolina

Toxicity tests with fathead minnow and Daphnia magna were conducted on-site at the International Paper plant in South Carolina during the period March 19-April 3, 1979. The Georgetown complex is considered, in the paper mill industry, as being a bleached-kraft, semi-chemical paper mill. The samples of "untreated" influent were collected from an open canal which collected the outfall from each mill process, i.e. primary clarifier, kraft pulping, bleach plant, cooling tower blowdown, tall oil, caustic waste and lime mud waste, prior to chemical treatment. The exact size of sample collection was just prior to the chemical treatment weir. The "treated" wastewater is then pumped

to a large aeration pond prior to being pumped into a retention/settling pond. The samples of "treated" effluent were collected at the pumping gate of the retention pond where the effluent was discharged into a canal which emptied into the Sampit River and subsequently Winyah Bay. Discharge of the effluent was conducted twice a day to correspond with the high ebb in the tidal zone. A flow diagram of the wastewater treatment process is presented in Figure 4.

The results of the acute toxicity studies with fathead minnow and daphnids exposed to the "untreated" influent and "treated" effluent are presented in Table 28. These data indicate 2.8 and 1.7 lethal units, respectively, were present in the "untreated" influent as determined from tests with fathead minnow and Daphnia magna. No mortality was observed in any of the tests with fathead minnows exposed to the treated effluent. In the 10 tests performed with daphnids and the effluent, none of the test concentrations produced more than 7% mortality. A 100% reduction in lethal units was provided for both the fathead minnow and Daphnia magna by the wastewater treatment process.

The results of the water quality parameters for the dilution water, influent and effluent are tabularized in Table 29. These data suggest that minimal variations occurred within each wastewater.

A summary of the water quality parameters measured in the exposure aquaria of the high, middle and low test concentrations including the control during the performance of the tests with both species are presented in Tables 30 and 31. These data indicate very minimal variations in the pH of each wastewater and that the depletion of the dissolved oxygen concentration in each test followed similar patterns observed at other sites and was directly related to test concentration. Specific conductance also varied in direct proportion to the test concentration.

Samples for the Ames Test were omitted at this test site at the request of the Project Officer.

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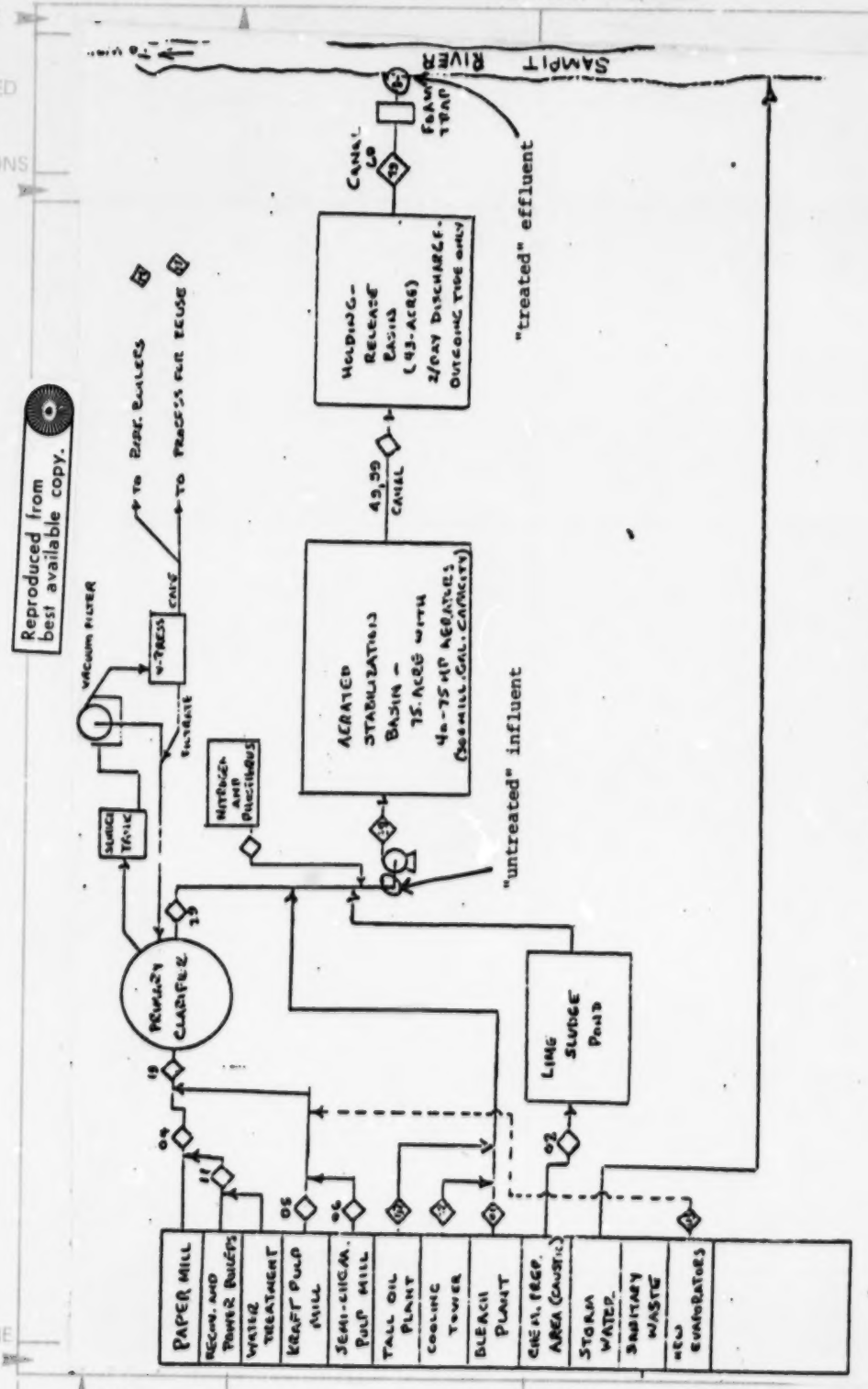

FIGURE 4. FLOW DIAGRAM FOR WASTE TREATMENT FACILITY AT INTERNATIONAL PAPER, GEORGETOWN, SOUTH CAROLINA.

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SECTION 4

DISCUSSION

On-site 24-hour static acute toxicity studies were performed on the wastewaters from 5 pesticide manufacturing plants, an organo-chemical manufacturer and a bleached kraft paper mill plant. The majority of the wastewater treatment systems evaluated consisted of the following (generalized) treatment steps, coarse screening of particulates, pH neutralization, activated sludge digestion and clarification. The findings generated by the contract will provide useful and relavent information both to industry and Federal and State regulatory agencies.

In the present study, the results of toxicity studies indicate a higher level of lethal units present in the wastewaters of the pesticide formulation, both before and after treatment as compared to the wastewaters of the pulp and paper mill and organo-chemical plant. A sufficient data base is not available to determine whether these observations are valid overall for the industries of concern. The average efficiency of the wastewater treatment plants in reducing the toxicity of the "untreated" wastewater was 98% (range, 92-100%). Excluded in the average were Mobay Chemical Company and E.I. duPont. Mobay was excluded since influent tests were not performed and consequently the percent efficiency of the treatment system could not be determined. E.I. duPont was excluded from the calculation since a true representation of the toxicity of the entire influent wastewater load could not be determined.

The results of ten consecutive days of biological testing at each site indicate that the toxicity of the "treated" effluent samples remained relatively constant from day to day. The average number of lethal units present in the "treated" effluents, as determined from tests with fathead minnows, was 2.1 with a range of 0-6 lethal units. Similarly, Daphnia magna tests provide a mean of 6.5 lethal units with a range of 0-34. As would be expected the toxicity of the "untreated" influent samples was extremely variable on a day to day basis. From the fathead minnow studies, the "untreated" influent contained a mean of 93 lethal units (range, 4.6-373), while Daphnia magna acute tests produced a mean of 261 lethal units (range, 1.7-782).

It is interesting to note that the toxicity of the "treated"

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effluent is relative to the toxicity of the "untreated" influent. For example, if an influent contained 300 lethal units and underwent biological and/or chemical treatment which has an efficiency of 99%, then the "treated" effluent would still contain 3 lethal units. This would be equal to an LC50 value of 33% effluent. Conversely, if an influent having moderate toxicity is subjected to the same treatment efficiency, the resulting effluent will probably be non-toxic or of a very low toxicity. The significance of this observation must be viewed in terms of what additional engineering steps have been applied to the effluent between the time the wastewater leaves the treatment system and enters the receiving water. Dilution with other treated process streams and non-contact cooling water must be taken into consideration in both designing wastewater treatment system and regulating their discharge.

The Mass Emission Rate as proposed by Esvelt et al. provides a useful method of incorporating lethal units and flow rates in assessing the impact of a wastewater stream on the total ecology of the receiving water. To determine the mass emission rate for a stream, one must first determine the relative toxicity (RT) of the wastestream which is defined as the product of the toxicity concentration (expressed as lethal units) and the flow rate.

$$RT = Tc \times \text{Flow Rate}$$

The mass emission rate then for a receiving water (Tc_2) is defined as the relative toxicity divided by the combined waste and dilution water flow.

$$Tc_2 = \frac{RT}{\text{wastewater flow rate} + \text{dilution water flow rate}}$$

At present, the mass emission rate for a receiving water is a somewhat nebulous value requiring further regulatory definition. If a program for water quality management is to be developed, which considers the toxic properties of wastes, then additional information must be obtained on the efficiency of the wastewater treatment systems. The utilization of a study program similar to that carried out under the present contract is one method by which the minimum efficiency of a wastewater treatment plant, under normal operating conditions, could be determined. If the toxicity of an effluent could be correlated with one or more chemical parameters which can be monitored continuously, then a mechanism would evolve by which regulators and plant operators could monitor the discharge of toxic wastes on a real time basis before they had a chance to cause damage to the environment.

Of the two species tested, neither the fathead minnow nor the water flea proved to be routinely more sensitive than the other in tests with the "untreated" or "treated wastewaters.

Since an adequate data base has not been developed for these species to the vast majority of industrial effluents, it is suggested that both the fathead minnow and daphnids be tested. On the other hand, should an on-going biomonitoring program be instituted for an industrial discharge in which both species provide similar results, then future testing might be limited to only one species. Daphnia magna are attractive for this purpose from the standpoint that their use can greatly reduce the economic burden to an industry for such a program. Daphnids are relatively easy to culture, require no capital investment and can be used by plant personnel to generate required data. It may also be assumed that with such a biomonitoring tool available to them, industry will take it upon themselves to incorporate its use on a regular basis in monitoring the efficiency of their wastewater treatment systems.

Finally, the use of the lethal unit concept should be applied to all effluent toxicity studies. LC50 values as percent effluent do not provide an accurate assessment of the impact of the wastewater. This is especially true for highly toxic wastes. The mean of a number of LC50 tests may not equal the mean from the same tests expressed as lethal units. A highly toxic effluent when converted to toxic units will skew the mean upwards and as a result will more accurately represent the toxic load entering the environment.

For those effluents resulting in less than 50% mortality in 100% effluent, the most probable toxicity concentration (expressed as lethal units) is equally important in determining mass emission rates.

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SECTION 5

REFERENCES

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SECTION 6

TABLES

TABLE 1. LIST OF THE SEVEN INDUSTRIAL WASTEWATER TREATMENT FACILITIES WHICH WERE CHOSEN FOR ON-SITE EFFLUENT TOXICITY STUDIES.

Modified list of sites	Dates of on-site testing	Ames test
Union Carbide, S. Charleston, W. Virginia	9/27/78-10/15/78	no
Monsanto, Muscatine, Iowa	10/17/78-11/2/78	yes
Mobay Chemical, Kansas City, Missouri	11/3/78-11/17/78	yes
Monsanto, Luling, Louisiana	12/1/78-12/17/78	yes
Diamond-Shamrock, Green Bayou, Texas	1/3/79-1/22/79	yes
duPont, LaPorte, Texas	2/8/79-2/27/79	no
International Paper, Georgetown, S. Carolina	3/20/79-4/2/79	no

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TABLE 2. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE SOUTH CHARLESTON, WEST VIRGINIA INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Untreated Influent																						
24-hour LC50 (% influent)	15	9.5	23	18	15	12	25	15	28	18	22	13	2.0	16	7.5	12	22	2.6	28	18	16	7
lethal units	6.7	10	4.3	5.6	6.7	8.3	4.0	6.7	3.6	3.6	4.5	7.7	50	6.3	13	8.3	4.5	38	3.6	5.6	10	12
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	
lethal units	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Daphnia magna - Untreated Influent																						
24-hour LC50 (% influent)	24	17	-*	-	26	21	-	-	19	14	-	-	1.8	13	-	-	50	4.7	-	-	19	13
lethal units	4.8	5.9	-	-	3.8	4.8	-	-	5.3	7.1	-	-	56	7.7	-	-	2.0	21	-	-	12	16
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	-
lethal units	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	-

*Tests not required at this time period.

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TABLE 3. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE SOUTH CHARLESTON, WEST VIRGINIA INDUSTRIAL WASTEWATER TREATMENT PLANT

	Diluent water		Treated effluent		Untreated effluent	
	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	22	21-23	22	18-24	27	24-31
pH	8.3	7.9-8.4	7.6	7.2-7.8	9.6	1.8-12
Dissolved oxygen (mg/ℓ)	NA*	NA	6.2	4.9-7.6	7.0	3.6-8.2
Conductivity (μmhos/cm)	470	440-500	1800	1400-2300	2600	700-7000
Hardness (mg/ℓ)	170	160-170	NA	NA	NA	NA
Alkalinity (mg/ℓ)	110	110-120	NA	NA	NA	NA
Chlorine (mg/ℓ)	ND†	ND	ND	ND	ND	ND

* Analyses not routinely performed.

† ND = non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 4. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE SOUTH CHARLESTON, WEST VIRGINIA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

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Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	7.9-9.1	7.8-9.1	8.2-8.4	8.2-8.4	440-470	420-480
	24	6.8-7.9	6.8-8.0	8.0-8.2	8.0-8.2	NA*	NA
42-7.5%†	0	8.1-9.2	6.5-8.7	3.8-11	9.1-11	500-3100	440-1100
	24	0.4-7.7	0.2-7.3	4.0-10	8.8-10	NA	NA
18-3.2%†	0	8.1-9.0	7.7-8.8	5.8-10	8.9-10	470-1700	480-730
	24	0.3-6.0	0.3-6.6	6.6-8.6	8.8-9.5	NA	NA
7.5-1.3%†	0	7.9-8.9	7.8-8.8	6.8-9.6	8.5-9.5	450-1000	460-600
	24	0.4-6.6	0.5-7.6	6.9-9.2	8.0-9.1	NA	NA
Effluent							
control	0	8.1-9.0	7.8-9.0	8.2-8.4	8.1-8.4	440-490	440-480
	24	6.2-8.2	7.0-8.1	8.0-8.2	8.0-8.2	NA	NA
100%	0	5.9-7.9	5.1-7.6	7.2-7.8	7.2-7.8	1400-2400	1400-2300
	24	3.8-6.8	3.9-6.7	7.4-8.0	7.4-8.0	NA	NA
42%	0	7.4-8.4	7.0-8.4	7.5-7.9	7.5-8.0	850-1200	850-1200
	24	5.2-7.5	5.7-7.3	7.6-8.0	7.6-8.0	NA	NA
18%	0	8.0-8.8	7.6-8.9	7.8-8.1	7.8-8.3	650-800	600-800
	24	6.0-7.7	6.7-7.8	7.8-8.1	7.9-8.1	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 5. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE SOUTH CHARLESTON, WEST VIRGINIA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	7.3-8.5	7.3-8.4	8.2-8.4	8.2-8.3	430-470	430-460
	24	7.3-8.2	7.2-8.2	8.2-8.3	8.0-8.3	NA*	NA
50-5.8%†	0	6.5-8.6	7.1-8.4	4.7-10	10-11	500-3200	600-1100
	24	5.7-8.2	2.5-7.8	4.8-9.3	8.9-9.4	NA	NA
21-2.5%†	0	7.1-8.6	7.2-8.4	6.4-10	9.2-10	495-1700	510-750
	24	3.4-6.4	0.3-6.2	7.2-9.2	8.3-9.0	NA	NA
9.0-1.0%†	0	7.2-8.8	7.1-8.5	7.0-9.7	9.0-9.6	470-800	470-600
	24	2.1-7.3	1.6-7.1	7.6-8.9	8.1-8.8	NA	NA
Effluent							
control	0	7.6-8.2	7.1-8.4	8.2-8.3	8.2-8.3	420-460	430-470
	24	7.3-8.4	7.0-8.2	8.2-8.3	8.1-8.3	NA	NA
100%	0	5.9-7.2	4.9-7.3	7.2-7.8	7.2-7.7	1400-2000	1400-2100
	24	6.1-7.9	6.0-7.4	7.9-8.4	7.8-8.4	NA	NA
42%	0	7.1-7.9	6.6-8.0	7.6-8.0	7.6-7.9	850-1100	860-1200
	24	7.0-8.3	6.8-7.8	8.1-8.4	8.0-8.4	NA	NA
18%	0	7.4-8.1	7.0-8.3	8.0-8.1	7.9-8.1	610-700	610-750
	24	7.0-8.4	7.0-8.0	8.2-8.3	8.1-8.3	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 6. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, MUSCATINE, IOWA INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Untreated Influent																						
24-hour LC50 (% influent)	<1.8	4.2	2.2	<1.8	3.4	1.4	2.1	2.2	1.5	2.2	1.8	4.2	<.05	2.2	1.5	2.2	2.2	2.2	1.5	2.7	2.3	0.85
lethal units	>56	24	45	56	29	71	48	45	67	45	56	24	200	45	67	45	45	45	67	37	47	14
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	81	81	56	65	65	81	81	81	81	52	52	52	52	65	65	81	65	65	65	81	68	12
lethal units	1.2	1.2	1.8	1.5	1.5	1.2	1.2	1.2	1.2	1.9	1.9	1.9	1.9	1.5	1.5	1.2	1.5	1.5	1.5	1.2	1.5	0.3
Daphnia magna - Untreated Influent																						
24-hour LC50 (% influent)	<1.8	2.7	-*	-	6.5	1.7	-	-	3.1	2.0	-	-	2.3	2.7	-	-	3.4	1.4	-	-	2.9	1.5
lethal units	>56	37	-	-	15	59	-	-	32	50	-	-	43	37	-	-	29	71	-	-	41	17
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	65	88	-	-	>100	85	-	-	78	78	-	-	64	73	-	-	65	81	-	-	75	9.0
lethal units	1.5	1.1	-	-	0	1.2	-	-	1.3	1.3	-	-	1.6	1.4	-	-	1.5	1.2	-	-	1.3	0.2

*Tests not required at this time period.

TABLE 7. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE MONSANTO, MUSCATINE, IOWA INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent		Untreated effluent	
	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	23	22-27	27	26-29	30	24-34
pH	8.4	8.3-8.4	7.3	7.2-7.4	5.6	4.6-9.1
Dissolved oxygen (mg/ℓ)	NA*	NA	6.6	6.0-7.6	7.2	6.6-8.1
Conductivity (μmhos/cm)	560	480-750	5200	4700-5800	5000	2600-12000
Hardness (mg/ℓ)	170	160-190	NA	NA	NA	NA
Alkalinity (mg/ℓ)	120	120-130	NA	NA	NA	NA
Chlorine (mg/ℓ)	-†	0.01-0.03	ND‖	ND	-†	0.03-10

* Analyses not routinely performed.

† Only 2 positive Cl_2 readings out of 20 readings.

‖ ND - non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 8. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, MUSCATINE, IOWA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF TESTING.

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Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.6-9.0	8.6-9.1	8.2-8.4	8.2-8.4	470-680	480-650
	24	7.2-8.2	7.7-8.5	8.2-8.3	8.2-8.4	NA*	NA
10-6.5%†	0	8.6-9.2	8.7-9.2	6.5-8.8	7.2-7.9	620-1600	640-950
	24	0.2-8.4	0.2-8.7	6.8-8.7	7.0-7.9	NA	NA
4.2-2.7%†	0	8.6-9.2	8.7-9.2	7.3-8.7	7.6-8.2	540-760	570-710
	24	0.2-6.4	0.4-8.6	7.2-8.4	7.2-8.0	NA	NA
1.8-0.49%†	0	8.4-9.1	8.6-9.1	8.0-8.5	7.9-8.3	500-600	520-690
	24	2.5-6.4	4.6-8.4	7.5-8.1	7.5-8.2	NA	NA
Effluent							
control	0	8.7-9.1	8.6-9.0	8.2-8.4	8.2-8.4	480-720	470-650
	24	7.4-8.4	7.8-8.4	8.2-8.3	8.2-8.4	NA	NA
100%	0	6.6-7.6	6.4-7.4	7.3-7.4	7.2-7.8	4400-5200	4400-5300
	24	5.7-7.4	6.1-8.3	7.6-7.7	7.1-7.8	NA	NA
42%	0	8.0-8.6	8.0-8.6	7.5-7.6	7.5-8.2	2000-2400	2300-2500
	24	6.6-7.7	7.1-7.9	7.7-7.8	7.8-7.9	NA	NA
18%	0	8.6-8.9	8.5-9.0	7.8-7.9	7.8-8.4	1300-1500	1300-1400
	24	7.2-8.1	7.6-8.2	7.9-8.0	8.0-8.1	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on toxicity results from the previous day.

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TABLE 9. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, MUSCATINE, IOWA INDUSTRIAL WASTE-WATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.6-8.8	8.6-8.9	8.3-8.4	8.4	480-670	480-650
	24	8.0-8.4	8.2-8.8	8.2-8.4	8.2-8.4	NA*	NA
10-4.2%†	0	8.6-8.9	8.5-9.1	7.5-8.8	7.6-8.2	650-980	550-780
	24	1.7-8.2	2.5-6.4	7.4-8.6	7.4-8.0	NA	NA
4.2-1.8%†	0	8.6-8.9	8.6-9.1	7.8-8.7	8.0-8.3	580-750	530-720
	24	4.8-8.0	5.1-6.8	7.6-8.4	7.7-8.1	NA	NA
1.8-0.75%†	0	8.6-8.9	8.2-9.0	8.1-8.5	8.0-8.3	540-680	510-680
	24	5.4-7.6	6.8-7.8	7.5-8.4	7.8-8.3	NA	NA
Effluent							
control	0	8.6-8.8	8.6-8.9	8.3-8.4	8.3-8.4	480-670	460-640
	24	8.0-8.4	8.1-8.7	8.2-8.4	8.2-8.4	NA	NA
100%	0	6.7-7.8	6.7-7.5	7.4	7.2	4300-5100	4400-4800
	24	7.6-8.0	7.4-8.2	8.0-8.1	8.0-8.1	NA	NA
42%	0	7.8-8.3	8.0-8.4	7.6	7.6-7.7	2200-2500	2200-2500
	24	8.0-8.3	7.9-8.5	8.1-8.2	8.1-8.2	NA	NA
18%	0	8.1-8.6	8.4-8.8	7.8-8.1	7.8-8.0	1200-1300	1200-1300
	24	8.2-8.5	8.0-8.8	8.2-8.3	8.2	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 10. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "TREATED EFFLUENT" FROM THE MOBAY CHEMICAL, KANSAS CITY, MISSOURI INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	32	26	40	32	40	32	40	32	26	26	32	26	26	26	21	17	21	17	21	23	28	7.0
lethal units	3.1	3.8	2.5	3.1	2.5	3.1	2.5	3.1	3.8	3.8	3.1	3.8	3.8	3.8	4.8	5.9	4.8	5.9	4.8	4.3	3.8	1.0
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	21	21	-*	-	26	20	-	-	26	22	-	-	23	26	-	-	23	21	-	-	23	2.4
lethal units	4.8	4.8	-	-	3.8	5.0	-	-	3.8	4.5	-	-	4.5	3.8	-	-	4.3	4.8	-	-	4.4	0.5

*Tests not required at this time period.

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TABLE 11. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE MOBAY CHEMICAL, KANSAS CITY, MISSOURI INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent	
	Mean	Range	Mean	Range
Temperature	22	22-24	24	20-26
pH	8.4	8.3-8.5	7.3	7.0-7.4
Dissolved oxygen (mg/ℓ)	NA*	NA	7.6	7.0-8.2
Conductivity (μmhos/cm)	500	500-550	21,000	20,000-23,000
Hardness (mg/ℓ)	170	160-180	NA	NA
Alkalinity (mg/ℓ)	120	120-130	NA	NA
Chlorine (mg/ℓ)	ND†	ND	ND	ND

*Analyses not routinely performed.

†ND = non-detectable, e.g. below detectable limits of 0.01 mg/ℓ.

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TABLE 12. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING ON-SITE TESTING OF FATHEAD MINNOWS* AND DAPHNIA MAGNA† TO "TREATED EFFLUENT" FROM THE MOBAY CHEMICAL, KANSAS CITY, MISSOURI INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TESTING PERIOD.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Effluent/Fathead minnow							
control	0	8.4-9.4	8.4-9.6	8.4-8.5	8.3-8.5	500-550	500-560
	24	7.6-8.6	7.7-8.7	8.2-8.4	8.2-8.4	NA[Π]	NA
50%	0	7.8-9.0	7.4-8.9	7.2-7.5	7.2-7.6	11000-12000	10000-12000
	24	0.4-3.9	0.4-4.5	7.5-7.7	7.6-7.8	NA	NA
21%	0	8.4-9.5	8.3-9.4	7.4-7.6	7.3-7.7	4500-5400	5000-6000
	24	2.7-6.5	1.3-6.7	7.6-7.8	7.6-7.8	NA	NA
8.9%	0	8.4-9.6	8.4-9.5	7.7-7.9	7.6-8.0	2200-2600	2100-2700
	24	5.0-7.8	4.7-8.0	7.8-8.0	7.7-8.0	NA	NA
Effluent/Daphnia magna							
control	0	8.4-9.1	8.4-9.0	8.4-8.5	8.3-8.4	500-550	500-550
	24	7.8-8.6	7.7-8.7	8.2-8.4	8.2-8.4	NA	NA
50%	0	7.7-8.8	8.0-8.5	7.2-7.6	7.2-7.6	11000-12000	10000-12000
	24	4.2-6.1	3.7-5.9	8.1-8.2	8.1	NA	NA
21%	0	8.2-9.1	8.3-8.9	7.4-7.7	7.4-7.7	5000-5500	5000-5500
	24	5.9-7.7	6.4-7.6	8.2-8.3	8.2	NA	NA
8.9%	0	8.2-9.2	8.4-9.0	7.7-8.0	7.8-7.9	2400-2500	2400-2500
	24	7.5-8.4	7.4-8.5	8.2-8.3	8.2	NA	NA

*Fathead minnows tested for ten consecutive days.

†Daphnia magna tested every other day, i.e. five days.

ΠAnalyses not routinely performed.

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TABLE 13. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, LULING, LOUISIANA INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Untreated Influent																						
24-hour LC50 (% influent)	<1.8	<1.8	1.0	0.6	1.5	1.2	1.2	1.5	1.5	1.8	1.0	1.0	1.8	1.8	1.8	1.5	2.2	1.5	1.8	2.2	1.5	0.4
lethal units	>56	>56	100	170	67	83	83	67	67	56	100	100	56	56	56	67	45	67	56	45	74	29
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	13	12	17	13	17	13	17	14	18	14	21	13	21	17	21	21	26	21	26	21	18	4
lethal units	7.7	8.3	5.9	7.7	5.9	7.7	5.9	7.1	5.6	7.1	4.8	7.7	4.8	5.9	4.8	4.8	3.8	4.8	3.8	4.8	6.0	1.5
Daphnia magna - Untreated Influent																						
24-hour LC50 (% influent)	1.8	1.3	-*	-	1.0	0.8	-	-	0.6	1.4	-	-	1.5	1.2	-	-	1.5	1.5	-	-	1.3	0.4
lethal units	56	77	-	-	100	120	-	-	170	71	-	-	67	83	-	-	67	67	-	-	88	34
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	32	32	-	-	40	32	-	-	25	21	-	-	40	44	-	-	32	36	-	-	33	7.0
lethal units	3.1	3.1	-	-	2.5	3.1	-	-	4.0	4.8	-	-	2.5	2.3	-	-	3.1	2.8	-	-	3.1	0.7

*Tests not required at this time period.

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TABLE 14. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE MONSANTO, LULING, LOUISIANA INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent		Untreated effluent	
	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	22	21-23	16	12-26	30	18-44
pH	8.3	8.1-8.5	8.6	8.4-8.7	12	11-13
Dissolved oxygen (mg/ℓ)	NA*	NA	9.7	8.4-11	5.9	0.4-9.4
Conductivity (μmhos/cm)	500	480-510	7900	6300-9000	21000	8300-67000
Hardness (mg/ℓ)	160	160-170	NA	NA	NA	NA
Alkalinity (mg/ℓ)	120	110-120	NA	NA	NA	NA
Chlorine (mg/ℓ)	ND†	ND	ND	ND	ND	ND-2.9

* Analyses not routinely performed.

† ND = non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 15. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOW TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, LULING, LOUISIANA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.7-11	8.5-10	8.1-8.4	8.0-8.5	460-510	460-500
	24	7.6-8.6	8.0-8.8	8.0-8.4	8.0-8.2	NA*	NA
10-2.7%†	0	8.2-11	7.9-10	9.5-11	9.3-12	680-1500	570-2200
	24	4.4-9.1	6.9-9.1	9.1-11	9.2-11	NA	NA
4.2-1.2%†	0	8.8-11	8.6-10	9.1-10	9.0-11	570-890	550-1100
	24	2.7-8.7	1.1-9.0	8.7-10	8.5-11	NA	NA
1.8-0.51%†	0	8.7-11	8.6-10	8.8-9.7	8.7-10	520-620	510-710
	24	2.6-8.1	1.6-8.8	8.4-9.2	8.6-10	NA	NA
Effluent							
control	0	8.6-11	8.7-10	8.2-8.6	8.0-8.6	450-570	460-500
	24	7.8-8.8	8.1-8.7	8.0-8.5	8.0-8.2	NA	NA
32%	0	8.8-11	8.6-11	8.5-8.7	8.5-9.9	3200-4400	3200-3600
	24	0.4-7.1	0.5-7.6	8.4-8.6	8.4-8.6	NA	NA
14%	0	8.8-11	8.8-11	8.5-8.7	8.5-9.4	1700-2200	1300-2000
	24	5.4-8.5	3.9-8.4	8.4-8.6	8.4-8.6	NA	NA
5.8%	0	8.7-11	8.6-10	8.4-8.6	8.4-9.0	1700-2200	1300-2000
	24	6.9-8.7	6.6-8.8	8.4-8.5	8.2-8.5	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 16. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE TESTING OF DAPHNIA MAGNA TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE MONSANTO, LULING, LOUISIANA INDUSTRIAL WASTE-WATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.7-9.9	8.6-9.5	8.0-8.4	8.0-8.4	450-550	470-490
	24	7.7-8.7	8.0-8.8	8.1-8.4	8.1-8.2	NA*	NA
6.5-27%†	0	8.5-9.6	7.9-9.6	9.6-11	9.8-11	800-1100	700-1400
	24	5.8-8.6	7.8-8.9	9.0-9.8	9.2-10	NA	NA
2.7-0.75%†	0	8.7-9.6	8.6-9.6	9.2-9.9	9.2-10	600-800	600-800
	24	4.4-8.4	1.8-8.6	8.0-9.0	8.5-9.5	NA	NA
1.2-0.32%†	0	8.6-9.7	8.6-9.7	8.7-9.5	8.6-9.4	500-600	500-600
	24	4.6-8.5	6.8-8.8	8.1-8.4	8.2-8.8	NA	NA
Effluent							
control	0	8.5-9.9	8.7-9.7	8.1-8.4	8.0-8.4	450-500	460-510
	24	7.7-8.7	8.1-8.9	8.1-8.2	8.1-8.4	NA	NA
77-50%†	0	8.4-9.9	8.4-9.7	8.5-8.6	8.4-8.6	4400-7000	4300-7000
	24	4.8-7.6	2.6-7.6	8.5-8.6	8.5-8.6	NA	NA
32-21%†	0	8.5-9.8	8.6-9.6	8.5-8.6	8.5-8.6	2300-3300	2200-3600
	24	7.0-8.4	6.0-8.6	8.5-8.6	8.4-8.6	NA	NA
14-8.9%†	0	8.7-9.8	8.7-9.6	8.5-8.6	8.4-8.6	1200-1800	1200-1800
	24	7.3-8.5	7.5-8.7	8.4-8.5	8.4-8.5	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 17. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE DIAMOND SHAMROCK, GREEN BAYOU, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Untreated Influent																						
24-hour LC50 (% influent)	>0.25	0.13	2.7	0.10	5.2	5.0	2.1	<0.045	8.1	5.2	0.25	4.2	3.4	4.2	0.06	0.06	5.2	4.2	<0.045	<0.17	3.3	2.4
lethal units	<400	770	37	1000	19	20	48	>2200	12	19	<400	24	29	24	1700	1700	19	24	>2200	>590	360	640
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	81	81	81	81	94*	94*	81	90*	81	90*	94*	90*	81	81	81	81	81	81	81	81	84	5.0
lethal units	1.2	1.2	1.2	1.2	1.1	1.1	1.2	1.1	1.2	1.1	1.1	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.0
Daphnia magna - Untreated Influent																						
24-hour LC50 (% influent)	0.20	<0.057	†	-	>0.49	>1.2	-	-	3.9	2.4	-	-	1.0	1.4	-	-	>4.2	3.6	-	-	2.1	1.3
lethal units	500	>1800	-	-	< 200	<80	-	-	26	42	-	-	100	71	-	-	<24	28	-	-	130	180
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	32	40	-	-	40	40	-	-	40	40	-	-	40	32	-	-	40	40	-	-	38	3.2
lethal units	3.1	2.5	-	-	2.5	2.5	-	-	2.5	2.5	-	-	2.5	3.1	-	-	2.5	2.5	-	-	2.6	0.2

*LC50 values calculated by plotting on graph paper.

†Tests not required at this time period.

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TABLE 18. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE DIAMOND-SHAMROCK, GREEN BAYOU, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent		Untreated influent	
	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	22	21-23	9.9	5.0-16	25	12-34
pH	8.2	8.0-8.5	7.8	7.2-8.0	7.1	2.7-12
Dissolved oxygen (mg/ℓ)	NA*	NA	10	7.8-12	7.3	3.0-9.4
Conductivity (μmhos/cm)	490	480-500	17000	12000-21000	25000	6000-64000
Hardness (mg/ℓ)	160	160-180	NA	NA	NA	NA
Alkalinity (mg/ℓ)	120	110-120	NA	NA	NA	NA
Chlorine (mg/ℓ)	ND†	ND	ND	ND	ND	ND

*Analyses not routinely performed.

†ND = non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 19. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE DIAMOND SHAMROCK, GREEN BAYOU, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

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Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.6-11	8.5-10	7.9-8.5	8.0-8.4	450-550	460-500
	24	6.6-8.0	7.3-8.7	7.9-8.3	7.2-8.4	NA*	NA
10-0.25%†	0	4.1-11	8.1-10	7.0-11	6.5-12	470-2900	500-6500
	24	0.5-8.9	6.8-8.9	7.1-9.9	6.9-11	NA	NA
4.2-0.10%†	0	7.5-11	8.7-10	7.5-9.5	7.0-11	460-1300	480-3200
	24	3.0-8.5	6.7-8.8	7.5-8.6	7.3-10	NA	NA
1.7-0.04%†	0	8.6-11	8.8-10	7.9-9.2	7.5-9.8	460-800	460-1400
	24	6.8-8.3	7.5-8.8	7.8-8.4	7.6-9.4	NA	NA
Effluent							
control	0	8.0-9.3	8.5-9.6	7.8-8.4	8.1-8.4	470-550	450-500
	24	7.2-8.3	7.4-8.5	7.8-8.4	7.9-8.4	NA	NA
100%	0	9.0-12	9.0-11	7.4-7.9	7.4-8.3	19000-22000	20000-22000
	24	0.7-5.4	0.6-5.9	7.2-7.8	7.3-7.8	NA	NA
42%	0	8.5-10	8.7-10	7.5-8.1	7.6-8.3	9000-10000	10000-11000
	24	2.2-7.0	1.6-7.4	7.3-7.9	7.5-7.9	NA	NA
18%	0	8.3-9.6	8.7-10	7.6-8.2	7.7-8.3	4500-7000	4500-7000
	24	3.5-7.6	3.2-8.3	7.4-8.0	7.5-7.9	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 20. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE DIAMOND SHAMROCK, GREENS BAYOU, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.6-9.1	8.3-8.9	7.8-8.4	8.1-8.5	470-550	480-500
	24	6.5-8.6	7.9-8.9	8.3-8.4	8.2-8.4	NA*	NA
10-0.32%†	0	8.7-9.2	7.5-9.0	7.4-8.2	6.9-12	500-1700	600-4500
	24	6.7-8.7	6.9-8.8	7.9-8.3	7.5-10	NA	NA
1.8-0.13%†	0	8.7-9.0	8.3-8.9	7.8-8.2	7.4-10	500-850	490-1800
	24	6.6-8.6	7.9-8.8	8.2-8.4	7.8-9.5	NA	NA
0.75-0.09%†	0	8.6-9.1	8.3-8.9	7.8-8.2	7.8-9.5	500-600	470-750
	24	6.6-8.7	7.9-8.8	8.2-8.4	8.0-8.8	NA	NA
Effluent							
control	0	8.5-8.9	8.3-8.8	7.7-8.3	8.0-8.5	470-500	460-600
	24	6.6-8.8	7.9-8.7	8.1-8.5	8.1-8.4	NA	NA
77-50%	0	9.0-10	8.9-10	7.3-8.0	7.5-8.0	11000-17000	11000-17000
	24	4.5-8.1	3.9-7.6	7.6-8.0	7.6-8.0	NA	NA
32-21%	0	8.8-9.4	8.7-9.6	7.3-8.1	7.6-8.1	5500-8000	5000-8000
	24	6.3-8.5	6.6-8.5	7.8-8.1	7.8-8.0	NA	NA
14-8.9%	0	8.6-9.0	8.4-9.0	7.4-8.2	7.7-8.2	2500-3800	2500-3700
	24	6.6-8.8	7.4-8.7	7.9-8.2	7.9-8.1	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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(4-76)

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TABLE 21. LC50 VALUE AND LETHAL UNITS FOR FATHEAD MINNOWS EXPOSED TO TWO "TREATED INFLUENTS" AND A "TREATED EFFLUENT" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Day/ 1		2		3		4		5		6		7		8		9		10			
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	Mean	St. Dev.
Fathead Minnow - Treated Influent*																						
24-hour LC50 (% influent)	26	26	32	40	32	<8.9	14	<8.9	32	>50	<8.9	8.9	32	11	40	32	<8.9	<8.9	26	<8.9	27	10
lethal units	3.8	3.8	3.1	2.5	3.1	>11	7.1	>11	3.1	<2.0	>11	11	3.1	9.1	2.5	3.1	>11	>11	3.8	>11	4.5	2.8
Fathead Minnow - Treated Influent†																						
24-hour LC50 (% influent)	0.54	0.84	0.65	0.80	0.84	0.84	Π	-	-	-	-	-	-	-	-	-	-	-	-	-	0.75	0.13
lethal units	180	120	150	120	120	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	140	27
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	42	42	52	52	42	52	42	42	32	42	34	42	42	34	42	43	42	42	42	34	42	5.6
lethal units	2.4	2.4	1.9	1.9	2.4	1.9	2.4	2.4	3.1	2.4	2.9	2.4	2.4	2.9	2.4	2.3	2.4	2.4	2.4	2.9	2.4	0.3

*Process wastewater Stream #1 - pesticide/non-pesticide main process water.

†Process wastewater Stream #2 - Maneb/Methomyl production prior to chemical oxidation treatment.

ΠTesting terminated due to process shutdown.

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TABLE 22. LC50 VALUES AND LETHAL UNITS FOR DAPHNIA MAGNA EXPOSED TO TWO "TREATED INFLUENTS" AND A "TREATED EFFLUENT" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Daphnia magna - Treated Influent*																						
24-hour LC50 (% influent)	>21	>21	-†	-	16	<9.0	-	-	10	>50	-	-	40	36	-	-	< 40	40	-	-	28	14
lethal units	<4.8	<4.8	-	-	6.2	>11	-	-	10	<2.0	-	-	2.5	2.8	-	-	> 2.5	2.5	-	-	4.8	3.3
Daphnia magna - Treated Influent‖																						
24-hour LC50 (% influent)	0.16	0.17	-	-	0.15	0.08	-	-	--ϕ	--	-	-	--	--	-	-	--	--	-	-	0.14	0.04
lethal units	620	590	-	-	670	1200	-	-	--	--	-	-	--	--	-	-	--	--	-	-	770	310
Daphnia magna - Treated Effluent																						
24-hour LC50 (% effluent)	2.4	<4.2	-	-	3.3	2.8	-	-	0.85	4.2	-	-	4.8	5.2	-	-	6.3	9.3	-	-	4.4	2.5
lethal units	42	>24	-	-	30	36	-	-	120	24	-	-	21	19	-	-	16	11	-	-	35	31

*Process wastewater Stream #1 - pesticide/non-pesticide main process water.

†Tests not required at this time period.

‖Process wastewater Stream #2 - Maneb/methomyl production prior to chemical oxidation treatment.

ϕTesting terminated due to process shutdown.

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TABLE 23. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent		Treated influent*	
	Mean	Range	Mean	Range	Mean	Range
Temperature (^{o}C)	23	22-24	16	11-21	34	30-39
pH	8.2	8.1-8.5	8.0	7.6-8.8	8.1	2.7-12
Dissolved oxygen (mg/ℓ)	NA†	NA	8.8	7.3-11	6.4	2.7-10
Conductivity (μmhos/cm)	500	490-510	9800	6000-11000	8700	3900-21000
Hardness (mg/ℓ)	170	160-180	NA	NA	NA	NA
Alkalinity (mg/ℓ)	120	120-140	NA	NA	NA	NA
Chlorine (mg/ℓ)	ND‖	ND	ND	ND	20	0-44

* Process wastewater stream #1 - pesticide/non-pesticide main process water.

† Analyses not routinely performed.

‖ ND = non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 24. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO TWO "TREATED INFLUENTS" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ)		pH		Conductivity (μmhos/cm)	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Influent*							
control	0	7.9-9.7	8.0-9.5	8.2-8.4	8.2-8.5	500	500
	24	6.6-8.6	7.1-8.4	8.1-8.4	8.1-8.3	NA†	NA
50%	0	7.5-8.9	6.7-9.5	7.7-12	3.7-10	2100-9000	2000-5000
	24	0.2-8.3	0.6-8.2	7.6-12	3.5-10	NA	NA
21%	0	8.0-9.5	8.1-9.5	7.9-12	5.5-9.7	1200-4000	1200-2700
	24	0.4-8.6	0.6-8.4	7.7-11	5.7-9.6	NA	NA
8.9%	0	7.4-9.7	8.1-9.4	8.1-11	7.0-9.2	800-1900	700-1500
	24	2.8-8.6	0.9-8.0	7.8-11	7.3-9.1	NA	NA
Influent‖,#							
control	0	8.5-9.5	9.0-9.1	8.3-8.4	8.3-8.5	500	500
	24	6.6-8.6	6.6-7.6	8.1-8.4	8.1-8.2	NA	NA
2.0%	0	8.5-9.5	9.0-9.3	8.4-9.0	8.9-9.1	500-700	650-700
	24	0.5-8.0	1.1-1.2	8.3	8.2-8.4	NA	NA
0.84%	0	8.7-9.6	9.1-9.2	8.4-8.8	8.6-8.9	500-600	550-600
	24	1.5-8.3	3.7-3.9	7.9-8.3	8.0-8.2	NA	NA
0.36%	0	8.6-9.5	9.0-9.2	8.3-8.6	8.4-8.6	500	500-600
	24	6.1-8.6	5.9-6.3	8.2-8.4	5.9-6.3	NA	NA

*Treated influent Stream #1 - pesticide/non-pesticide main process water.

†Analyses not routinely performed.

‖Treated influent Stream #2 - Maneb/Methomyl production prior to chemical oxidation treatment.

#Testing terminated after day 3 due to process shutdown.

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TABLE 25. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO "TREATED EFFLUENT" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ)		pH		Conductivity (μmhos/cm)	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Effluent							
control	0	8.4-9.6	8.4-9.4	8.2-8.5	8.2-8.5	500	500
	24	6.7-8.3	6.7-8.0	8.2-8.4	8.1-8.4	NA*	NA
100%	0	7.8-11	7.9-10	7.8-8.1	7.8-8.2	10000-120000	10000-12000
	24	0.4-4.0	0.9-4.9	7.6-7.9	7.6-7.8	NA	NA
42%	0	8.3-9.7	8.5-10	7.9-8.2	7.9-8.2	4700-6000	5000-6000
	24	0.8-6.3	1.8-6.8	7.6-8.1	5.7-8.1	NA	NA
18%	0	8.4-9.7	8.5-9.6	8.0-8.3	8.0-8.3	2400-3000	2500-2900
	24	3.0-7.1	3.3-7.5	7.6-8.1	7.8-8.2	NA	NA

*Analyses not routinely performed.

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TABLE 26. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO TWO "TREATED INFLUENTS" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF ON-SITE TESTING.

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Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent*							
control	0	8.5-9.3	8.7-9.1	8.2-8.5	8.2-8.5	500	500
	24	7.8-8.7	7.6-9.4	8.2-8.4	8.2-8.4	NA†	NA
50-21%Π	0	7.7-8.9	7.7-9.4	7.9-12	3.7-9.2	1100-9500	1400-5500
	24	1.8-8.3	1.3-9.2	7.7-12	3.5-8.0	NA	NA
21-8.9%Π	0	8.3-9.3	8.3-9.7	8.0-12	5.6-8.9	950-3900	750-2700
	24	2.9-8.4	2.9-9.0	7.7-11	5.7-8.1	NA	NA
8.9-4.2%Π	0	8.5-9.3	8.6-9.3	8.1-11	7.3-8.7	600-1700	650-1500
	24	4.8-8.3	2.6-8.1	7.8-10	7.8-8.1	NA	NA
Influent#,§							
control	0	8.8-8.9	8.4-8.5	8.3-8.5	8.3-8.5	410-500	480-500
	24	7.1-7.8	7.8-8.0	8.1-8.4	8.1	NA	NA
0.32-0.21%Π	0	8.8-9.0	8.5-8.7	8.5-8.6	8.4-8.6	430-550	490-500
	24	7.6-7.8	7.2-7.9	8.2-8.3	8.0-8.2	NA	NA
0.13-0.09%Π	0	8.7-8.8	8.6	8.4-8.5	8.4-8.6	490-500	480-500
	24	6.9-8.0	7.9-8.1	8.2	8.1-8.2	NA	NA
0.06-0.04%Π	0	8.7-8.8	8.5-8.6	8.3-8.5	8.3-8.5	500	450-500
	24	7.8-8.0	7.8-7.9	8.2-8.4	8.1-8.2	NA	NA

*Treated influent Stream #1 - pesticide/non-pesticide main process water.

†Analyses not routinely performed.

ΠTreated influent Stream #2 - Maneb/Methomyl production prior to chemical oxidation treatment.

#Testing terminated after day 3 due to process shutdown.

§Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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TABLE 27. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO "TREATED EFFLUENT" FROM THE DUPONT, LAPORTE, TEXAS INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ)		pH		Conductivity (μmhos/cm)	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Effluent							
control	0	8.4-8.9	8.0-9.1	8.3-8.5	8.2-8.5	460-500	490-500
	24	7.1-8.3	6.5-7.9	8.1-8.4	8.0-8.3	NA*	NA
21%	0	8.4-9.4	8.4-9.3	8.0-8.2	7.9-8.2	2000-3500	2200-3000
	24	5.9-6.8	5.2-7.3	7.8-8.0	7.8-8.0	NA	NA
8.9%	0	8.4-9.3	8.4-9.3	8.1-8.4	8.0-8.3	1200-1700	1400-1600
	24	6.2-7.1	5.8-7.7	7.8-8.1	7.9-8.1	NA	NA
4.2%	0	8.3-9.3	8.2-9.0	8.2-8.4	8.1-8.4	800-1000	950-1000
	24	6.6-7.6	5.9-7.4	7.9-8.2	7.9-8.2	NA	NA

* Analyses not routinely performed.

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TABLE 28. LC50 VALUES AND LETHAL UNITS FOR FATHEAD MINNOWS AND DAPHNIA MAGNA EXPOSED TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE INTERNATIONAL PAPER, GEORGETOWN, SOUTH CAROLINA INDUSTRIAL WASTEWATER TREATMENT PLANT.

Day/	1		2		3		4		5		6		7		8		9		10		Mean	St. Dev.
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.		
Fathead Minnow - Untreated Influent																						
24-hour LC50 (% influent)	52	34	42	34	34	42	42	52	52	42	42	23	42	42	23	21	42	34	27	<12	38	9.5
lethal units	1.9	2.9	2.4	2.9	2.9	2.4	2.4	1.9	1.9	2.4	2.4	4.3	2.4	2.4	4.3	4.8	2.4	2.9	3.7	>8.3	2.8	0.8
Fathead Minnow - Treated Effluent																						
24-hour LC50 (% effluent)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	
lethal units	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Daphnia magna - Untreated Influent																						
24-hour LC50 (% influent)	>65	>43<65	-*	-	55	81	-	-	77	73	-	-	77	>65	-	-	41	42	-	-	64	17
lethal units	<1.5	>1.5 <4.2	-	-	1.8	1.2	-	-	1.3	1.4	-	-	1.3	<1.5	-	-	2.4	2.4	-	-	1.7	0.5
Daphnia magna - Treated Effluent†																						
24-hour LC50 (% effluent)	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	>100	-	-	>100	
lethal units	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	

*Tests not required at this time period.

†No mortalities observed in any test concentration.

TABLE 29. RESULTS OF WATER CHEMISTRY ANALYSES CONDUCTED ON 100% TEST WATERS DURING TEN CONSECUTIVE DAYS OF ON-SITE TESTING AT THE INTERNATIONAL PAPER, GEORGETOWN, SOUTH CAROLINA INDUSTRIAL WASTEWATER TREATMENT PLANT.

	Diluent water		Treated effluent		Untreated influent	
	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	22	21-23	21	17-23	37	35-41
pH	8.3	8.2-8.4	7.5	7.1-7.6	7.1	6.1-8.6
Dissolved oxygen (mg/ℓ)	NA*	NA	4.3	2.5-5.2	3.0	0.4-4.9
Conductivity (μmhos/cm)	500	500	2000	1800-2100	2200	1300-2600
Hardness (mg/ℓ)	170	170-180	NA	NA	NA	NA
Alkalinity (mg/ℓ)	120	110-130	NA	NA	NA	NA
Chlorine (mg/ℓ)	ND†	ND	ND	ND	ND	ND

* Analyses not routinely performed.

† Non-detectable, e.g. below detectable limit of 0.01 mg/ℓ.

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TABLE 30. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF FATHEAD MINNOWS TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE INTERNATIONAL PAPER, GEORGETOWN, SOUTH CAROLINA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE TEN CONSECUTIVE DAYS OF ON-SITE TESTING.

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Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.7-9.3	8.8-9.2	8.2-8.3	7.4-8.3	500	500
	24	7.8-8.6	8.1-8.5	8.2-8.3	7.7-8.2	NA*	NA
65%	0	4.7-7.5	3.9-7.9	6.7-8.1	6.7-8.4	1200-1600	1100-1500
	24	0.3-0.6	0.2-1.3	6.8-7.7	6.9-7.8	NA	NA
27%	0	7.3-8.5	7.4-8.5	7.1-8.1	7.2-8.1	750-980	750-900
	24	1.0-4.6	0.5-5.5	7.3-7.7	7.4-7.8	NA	NA
12%	0	8.3-9.2	8.2-9.0	7.4-8.2	7.6-8.1	600-700	600-700
	24	4.1-6.2	4.5-7.0	7.4-7.9	7.6-7.8	NA	NA
Effluent							
control	0	8.6-9.1	8.8-9.2	8.2-8.3	8.0-8.3	500	500
	24	8.1-8,6	8.0-8.6	8.2-8.3	8.1-8.2	NA	NA
100%	0	1.5-5.4	2.5-4.0	7.2-7.6	7.0-7.6	1900-2000	1800-2100
	24	0.3-0.8	0.3-0.8	7.3-7.6	7.2-7.5	NA	NA
42%	0	6.3-7.7	6.8-7.5	7.4-7.8	7.2-7.7	1100-1200	1100-1200
	24	2.5-4.0	2.6-4.2	7.4-7.6	7.3-7.6	NA	NA
18%	0	7.9-8.4	8.0-8.8	7.8-8.0	7.2-7.9	800-850	700-800
	24	5.8-6.7	6.3-7.3	7.6-7.8	7.4-7.8	NA	NA

* Analyses not routinely performed.

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TABLE 31. RESULTS OF WATER CHEMISTRY ANALYSES PERFORMED DURING THE ON-SITE TESTING OF DAPHNIA MAGNA TO "UNTREATED INFLUENT" AND "TREATED EFFLUENT" FROM THE INTERNATIONAL PAPER, GEORGETOWN, SOUTH CAROLINA INDUSTRIAL WASTEWATER TREATMENT PLANT. THESE VALUES REPRESENT THE RANGE OF MEASUREMENTS OBSERVED DURING THE FIVE DAYS OF ON-SITE TESTING.

Concentration	Hour	Dissolved oxygen (mg/ℓ) A.M.	Dissolved oxygen (mg/ℓ) P.M.	pH A.M.	pH P.M.	Conductivity (μmhos/cm) A.M.	Conductivity (μmhos/cm) P.M.
Influent							
control	0	8.6-9.2	8.5-9.0	8.2-8.3	8.2-8.3	500	500
	24	8.0-8.5	8.3-8.7	8.2	8.2	NA*	NA
100-65%†	0	4.6-7.1	2.6-7.0	6.8-7.4	6.5-7.7	1500-1900	1100-1800
	24	0.8-1.8	0.7-2.4	7.0-7.8	7.2-8.0	NA	NA
42-27%†	0	7.5-8.4	6.7-8.1	7.2-7.7	7.1-7.9	950-1100	800-1000
	24	1.9-5.9	3.6-5.5	7.7-7.9	7.5-7.9	NA	NA
18-12%†	0	8.2-9.2	7.8-8.4	7.6-8.0	7.5-8.0	700-800	600-750
	24	5.0-7.6	6.1-7.5	7.8-8.0	7.8-8.0	NA	NA
Effluent							
control	0	8.6-9.0	8.5-9.0	8.2-8.3	8.2-8.3	500	500
	24	8.0-8.4	8.3-8.6	8.2	8.2	NA	NA
100%	0	2.6-3.9	2.6-3.9	7.4-7.6	7.4-7.6	1800-2100	1700-2000
	24	2.3-3.5	2.6-3.7	7.8	7.7-7.8	NA	NA
42%	0	6.9-7.4	6.7-7.2	7.6-7.8	7.6-7.7	1100	1000-1100
	24	5.3-6.9	5.6-6.7	7.9-8.0	7.8-7.9	NA	NA
18%	0	7.9-8.5	8.0-8.4	7.8-7.9	6.7-7.9	750-800	700-800
	24	7.4-7.9	7.4-7.9	8.0-8.1	7.5-8.1	NA	NA

*Analyses not routinely performed.

†Test concentrations varied during testing and were based on results of toxicity data from the previous day.

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APPENDIX

EG&G MASON RESEARCH INSTITUTE

Modified Protocol for Salmonella/Mammalian Microsome Plate Incorporation Mutagenesis Assay

Media

Top agar is initially prepared with 13.4 g/liter Difco Bacto Agar and 8.4 g/liter NaCl. After autoclaving, the molten agar is distributed in 100 mℓ aliquots into sterile bottles where it is stored at room temperature. Immediately before its use in the mutagenesis assay, the top agar is melted and supplemented with 10 mℓ/100 mℓ agar of a sterile solution containing 0.8 mM L-histidine and 0.8 mM biotin (1XSA). Twenty-five mℓ of sterile deionized water is added per 100 mℓ top agar when it is used in assays without metabolic activation. This insures that final top agar and amino acid supplement concentrations are the same on plates with or without metabolic activation.

Top agar for cell titers is initially prepared with 8 g/liter Difco Bacto Agar and 5 g/liter NaCl. After autoclaving, the molten agar is distributed in 100 mℓ aliquots. Immediately before its use, the top agar is melted and supplemented with 10 mℓ/100 mℓ agar of a sterile solution containing 5.0 mM L-histidine and 0.5 mM biotin (10XSA).

Bottom agar is the Vogel-Bonner minimal medium E described by Ames.

Nutrient broth used for growing overnight cultures of the tester strains contains 25 g per liter of Nutrient Broth No. 2 (Oxoid).

Storage and Preparation of Tester Strains

All tester strains are stored in liquid nitrogen, and fresh cultures are inoculated directly from these frozen stocks. Broth cultures are grown overnight at 37°C with shaking. If necessary, the cultures are then centrifuged and resuspended in their culture medium to give an appropriate final cell concentration. Each culture is routinely checked for crystal violet sensitivity and ampicillin resistance before use in the mutagenesis assay.

Toxicity Determination of Test Compounds

Each test compound is checked for toxicity to the tester strains up to a concentration of 1.0 mℓ/plate. Eight serial half-log dilutions of the test compound are plated with TA100 on minimal agar plus 10XSA and on minimal agar plus 1XSA. Equal numbers of cells are seeded on each plate in the presence of test compound. The percent survival of an appropriately diluted TA100 culture on the 10XSA supplemented plates is determined by comparing numbers of colonies on the solvent control with those on the plates containing test compound. Toxicity of the 1XSA supplemented plates is detectable by a decrease in the number of revertant colonies occurring per bacterial lawn. The highest concentration of test compound used in the broad range mutagenesis assay is that which gives approximately 35% survival on the 10XSA supplemented plates and a detectable reduction of spontaneous revertants on the 1XSA supplemented plates. If no toxicity is apparent, then the highest concentration used in the broad range mutagenesis assay is 1.0 mℓ/plate.

Plating Procedures for Mutagenesis Plate Incorporation Assay

Routinely, the test compound is thawed and filtered immediately before its use in the mutagenesis assay. Five doses of the compound are first plated with all five tester strains (TA98, TA100, TA1535, TA1537, TA1538) with metabolic activation, after which they are immediately plated on all tester strains without metabolic activation. All positive controls, solvent controls, and test compound dilutions are plated in triplicate. Without metabolic activation, 50 uℓ of tester strain and 1.0 mℓ of solvent or test compound are added to 1.5 mℓ of molten top agar at 45°C. With metabolic activation, 50 uℓ of tester strain, 1.0 mℓ of solvent or test compound, and 0.5 mℓ of S-9 mix are added to 1.0 of molten top agar at 45°C. After vortexing, the mixture is poured onto the surface of 25 mℓ of bottom agar contained in a 15 x 100 mm plastic pertri dish.

Positive Controls

Positive controls are run with each day's assays. 2-Aminoanthracene (2AA) is plated at 0.4 ug/plate with metabolic activation on strains TA98, TA100, and TA1538. Propane sultone (PS) is plated at 0.02 uℓ/plate without activation on TA1535, and 9-aminoacridine (9AAD) is plated at 75 ug/plate on TA1537 without activation.

Tester Strain Titers

Tester strain titers are determined by viable count assays on 10XSA supplemented minimal agar plates. The number of cells plated per plate is reported on form WL-59 in the final report.

Preparation and Storage of Liver Microsomal Enzymes

Liver microsomal enzymes are routinely prepared from male Sprague-Dawley rats that have been injected with Arochlor 1254 at mg/kg. The Arochlor is diluted in corn oil to a concentration of 200 mg/mℓ. Five days after their i.p. injection with the Arochlor, the rats are sacrificed by decapitation, and their livers are excised. The rats are denied access to food for 12 hour immediately preceding sacrifice.

The preparation of the microsomal enzyme fraction is carried out with sterile glassware and solutions at 0-4°C. The liver from each rat is excised and placed in 20 m of 0.15 M KCl contained in a pre-weighed beaker. After weighing the liver, it is transferred to another beaker containing 3 volumes of 0.15 M KCl (3 mℓ/g of wet liver) where it is minced with sterile scissors. The minced liver is homogenized in a Potter-Elvehjen apparatus with a teflon pestle. The homogenate is centrifuged at 9000 x g for 10 minutes in the SS-34 rotor of a Sorvall SS-3 centrifuge. The supernatant (referred to by Ames at the S-9 fraction) is decanted, and small aliquots are distributed into freezing ampules which are stored in liquid nitrogen.

One mℓ of the microsomal enzyme reaction mixture (S-9 mix) which is added to the soft agar overlay contains the following components:

S-9	0.05 mℓ
0.4 M $MgCl_2$	0.02 mℓ
1.65 M KCl	0.02 mℓ
0.04 M NADP	0.10 mℓ
0.05 M Glucose-6-phosphate	0.10 mℓ
1.00 M NaH_2PO_4, pH 7.4	0.10 mℓ
H_2O	0.61 mℓ
	1.00 mℓ

Evaluation of Mutagenesis Assay Data

For a compound to be considered positive, it must cause a doubling in the observed reversion index of at least one tester strain. The increase in reversion index must be accompanied by a dose response to increasing concentrations of the test compound.